Medical Entomology Consultant
to the
U.S. Army Surgeon General

GUIDE TO
ENTOMOLOGICAL SURVEILLANCE
DURING
CONTINGENCY OPERATIONS

October 2001

GUIDE TO ENTOMOLOGICAL SURVEILLANCE DURING CONTINGENCY OPERATIONS

Foreword

This document consolidates information and procedures for surveillance of arthropod vectors during military deployments. In light of the amount of current research being conducted in this area, I fully expect the need to continually revise and update this information. Your constructive comments are most welcome and will be given full consideration in the updating of this document. Please contact:

Lieutenant Colonel Leon L. Robert, Jr., U.S. Army
Division of Tropical Public Health
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20814-4799
Phone: (301) 295-3733; DSN: 295-3733
Fax: (301) 295-3860; DSN: 295-3860
E-mail: lrobert@usuhs.mil

Endorsed by and distributed under the auspices of:

Colonel Daniel Strickman
Medical Entomology Consultant to the U.S. Army Surgeon General
Walter Reed Army Institute of Research
Washington, DC 20307-5100

Acknowledgments

LTC Leon L. Robert Jr. prepared this document in response to deployment and field experiences in the USEUCOM and USCENTCOM areas of responsibility. Many DoD military and civilian entomologists provided information, reviews, and valuable comments to ensure a complete and current document.

Disclaimer

Mention of a specific product, trade name, or manufacturer does not constitute an official endorsement of these products, but is intended for illustration and information purposes only.

TABLE OF CONTENTS

CHAPTER 1: Introduction

1. Background.

The Department of Defense has directed that joint military medical surveillance be conducted during all applicable military operations (DOD-Directive 6490.2, *Joint Medical Surveillance*, 30 Aug 97 and DOD-Instruction 6490.3, *Implementation and Application of Joint Medical Surveillance for Deployments*, 7 Aug 97). This encompasses all aspects of military preventive medicine that pertain to medical surveillance for major deployments identified by the Chairman of the Joint Chiefs of Staff in coordination with the Assistant Secretary of Defense for Health Affairs. Based upon DODI 6490.3, The Joint Chiefs of Staff have directed the Services to conduct a systematic and comprehensive program of surveillance, assessment, and prevention of occupational and environmental health hazards (JCS Memorandum MCM-251-98, Subject: *Deployment Health Surveillance and Readiness*, 4 Dec 98).

Medical surveillance, as defined by DOD-D 6490.2, is “the regular or repeated collection, analysis, and dissemination of uniform health information for monitoring the health of a population, and intervening in a timely manner when necessary.” The final link of a military medical surveillance system is the application of these data to military training, plans and operations to prepare and implement early intervention and control strategies. The surveillance system includes a functional capacity for timely data collection, analysis and dissemination of information linked to military preventive support of operational commanders.

Historically, infectious diseases have been responsible for four times more casualties than battle injuries (Joint Publication 4-02, “*Doctrine for Health Service Support in Joint Operations*, 30 Jul 01). They may cause significant numbers of casualties within the first 48 hours to first few weeks of a deployment. The anticipation, prediction, identification, prevention and control of vector-borne disease threats to military personnel and military units are a critical part of the Joint Medical Surveillance system. Whether engaged in armed conflict, or deployed in support of peacekeeping or humanitarian operations, commanders throughout the DOD are concerned about vector-borne disease threats, which could adversely affect the health of their troops. The DOD has recognized these concerns and taken a proactive approach to protect service members and civilians supporting contingency operations.

Decision-making is a key component in prevention of vector-borne diseases through timely surveillance and subsequent integrated pest management (IPM). In an IPM context, decision-making relies on protocols for deciding the need for some management action based on an assessment of the state of the pest population and subsequent potential for the transmission of vector-borne disease to humans. These protocols (also referred to as control decision rules) consist of

two components: (1) standardized procedures for assessing the density of the pest populations, and (2) an economic (=action) threshold. The action threshold in reference to vector-borne diseases is a practical or operational rule. It can be quantitatively defined as the lowest population density above an acceptable baseline value that will cause nuisance and/or disease. Many would agree that perhaps even one potentially infected vector is unacceptable. Assessment of pest density usually requires obtaining actual counts of pests, and therefore, sampling is critically important. Because sampling is time consuming, expensive and potentially delays control efforts, one must know how to gather enough information about pest abundance to be able to make correct decisions without taking excessive time or consuming excessive resources.

Decision-making in IPM is important for three reasons. First, decision-making protocols can be used to reduce pesticide use. Ideally, IPM relies on benign tactics such as good sanitation, habitat modification, and cultural practices to maintain fluctuating pest populations below nuisance and disease injury levels. The second reason why decision-making is important in IPM is that if tactics such as good sanitation and cultural practices fail, pest control can still be accomplished through the effective use of pesticides, and assessment and decision-making protocols must be available to determine when to intervene. Third, the risk assessment of the vector-borne disease threat obtained through entomological surveillance can influence decisions on the use of medical preventive interventions (e.g., chemoprophylaxis).

2. Purpose.

a. This document provides guidance for conducting baseline entomological assessments of proposed US Forces locations and entomological health surveillance of occupied US Forces locations in accordance with the intent of DOD Instruction 6490.3. The purpose of an entomological assessment is to rapidly identify entomological, health and safety hazards that pose potential risks to US personnel at proposed or occupied US Forces locations. This document represents the first guidance for conducting quantitative entomological assessments and an attempt to standardize entomological assessments within the Service components to better protect personnel during joint military deployments.

b. This document is intended for use by DOD enlisted and officer preventive medicine, public health, pest control, entomology, environmental science and environmental engineering personnel who are deployed with US Forces. It is assumed that the user of this information will have rudimentary baseline knowledge of military entomological practices gained from baseline (e.g., entry-level) preventive medicine training.

c. Entomological assessment techniques (e.g., descriptions of traps and sampling procedures) are described in sufficient detail to enable those with

limited access to scientific publications and other resources due to their deployment to understand and easily use the methods. In addition, guidance is given on preferred methods and possible alternative methods, should it become necessary to use them.

d. Although economic thresholds (=action thresholds) for most medically important insect pests have not been established or widely accepted, this document attempts to provide general thresholds (=rule of thumb) for many of the common insect pests and vectors of human disease. The economic threshold in reference to vector-borne diseases is a practical or operational rule. It can be quantitatively defined as the lowest population density that will cause nuisance and/or disease above an acceptable baseline value. Many would agree that perhaps even one case of some vector-borne diseases is unacceptable.

e. During military deployments, known and potential vector arthropods should be closely monitored and vector populations reduced when the tactical situation and resources allow. It is accepted that each deployment will be different in many ways (geographic location, vector-borne disease threat, mission, tactical environment, force protection rules, climate, etc.), which will require rapid decision-making and modification of entomological surveillance guidelines. However, accepted principles of IPM, to include entomological surveillance, must always be at least considered and implemented as appropriate, if the tactical situation permits. The operational entomology practitioner should always bear in mind that people in developing countries live in closer contact to their environment and are therefore even more susceptible to the harmful effects of misapplied pesticides than people in developed countries.

3. Notes On Preservation For Detection Of Pathogens.

The methods for detection of pathogens in arthropods are evolving very rapidly. Formerly, specimens had to be frozen on dry ice for this purpose. More recently, techniques have been developed for detection of malaria sporozoite antigen in dried anophelines held at room temperature. In 2001, a "dipstick" developed jointly by the military and industry will be field for detection of the sporozoite antigen. The tool is produced as a self-contained kit requiring no refrigeration. It produces a result in 15 minutes and distinguishes between *vivax* and *falciparum* malaria. A great number of tests have been developed based on detection of DNA or RNA or pathogens by amplification of specific sequences with PCR. Freezing of specimens on dry ice will always work, but many of the methods will also detect the pathogens preserved in 100% alcohol or even dried at room temperature. Of course, many of these preservation techniques are not compatible with morphological identification of the vector species. This places a greater burden on the collector to identify the specimens before they are tested.

CHAPTER 2: On-Site Entomological Assessments

1. **An initial comprehensive entomological survey should be conducted within at least the first 30 days of any deployment**. The extent and locations of this survey are dependent on the tactical situation, mission, geographic area involved, time of year, and threat of vector-borne disease. Early recognition of vectors of endemic diseases and other pests and appropriate countermeasures will reduce disease non-battle injuries and greatly contribute to mission success. The need and frequency of subsequent surveys is dependent on the results of the initial survey and changes over time in the overall situation.

2. Sampling of the general geographic site area should include at least all elements indicated in Table 1 below. Some of this information may have already been collected during previous assessments. It is important to seek as much general information about the physical location of the site as possible. This will include information about the geographic location of the site, administrative support available (i.e., communications, computer), logistical support available (i.e., supplies available, storage of supplies) and host unit support (i.e., lodging, food, water, workspace, transportation, storage).

3. In addition, general environmental health data, as it relates to entomology, should also be collected (Table 2). This includes information related to base camp drainage, work and living areas (e.g., trash, garbage and human waste disposal), endemic diseases, history of pesticide usage, hazardous materials, toxic industrial chemicals and industrial/agricultural activities that are nearby.

4. The entomology information in Table 3 relates specifically to pest management practices and vector surveillance and control activities. The collection of this information is essential in determining the extent of the pest management activities being conducted and ensuring that they are conducted in a safe and effective manner.

GENERAL ENTOMOLOGICAL ASSESSMENT CHECKLIST

Reviewer: _____
Organization: _____
Operation: _____
Date: _____

STATUS
(G)REEN – **Completed/Functioning**
(A)MBER – **Marginal** (Notes required)
(R)ED – **Insufficient** (Notes required)

TABLE 1. GENERAL ENVIRONMENTAL ASSESSMENT

A. General Setting **POC:** **Phone/Email:**

Status	No.	Required Elements	Field Notes (use reverse if needed)
G A R	1.	**# Personnel to be deployed**	
G A R	2.	**Anticipated duration of deployment**	
G A R	3.	**Physical location**	
G A R	a.	-grid coordinates [1]	
G A R	b.	-acreage	
G A R	c.	-level ground	
G A R	d.	-drainage	
G A R	e.	-soil consistency	
G A R	f.	-seasonal climactic effect (wind, rain, snow)	
G A R	g.	-will host nation allow personnel to dig?	
G A R	4.	**Communications**	
G A R	a.	-types of radios present[1]	
G A R	5.	**Computer**	
G A R	a.	-what type of computer support is available?	
G A R	b.	-number of terminals (MIPR or SIPR Net)	
G A R	c.	-LAN? [1]	
G A R	d.	-email capability?	
G A R	e.	-internet capability?	
G A R	6.	**Telephone support**	
G A R	a.	-secure (STU III) [2]	
G A R	b.	-non-secure (DSN/MSE) [2]	

TABLE 1. GENERAL ENVIRONMENTAL ASSESSMENT

A. General Setting **POC:** **Phone/Email:**

Status	No.	Required Elements	Field Notes (use reverse if needed)
G A R	c.	-FAX[2]	
G A R	d.	- Phone numbers? [2]	
G A R	e.	-Country code or local prefixes?	
G A R	7.	**Logistics**	
G A R	a.	-procedures for moving supplies[1, 2]	
G A R	b.	-procedures for moving samples (at site and receiving location) [1, 2]	
G A R	c.	-local available transportation[1, 2]	
G A R	d.	**-** Local sources of Dry Ice and Liquid Nitrogen?	
G A R	8.	**Host unit support**	
G A R	a.	-will host unit provide lodging?	
G A R	b.	-will host unit provide food?	
G A R	c.	-will host unit provide workspace?	
G A R	d.	-will host unit provide transportation?	
G A R	e.	-space for medical storage? [1, 2]	
G A R	9.	***Personal protective equipment***	
G A R	a.	-Foul weather gear	
G A R	b.	-NBC gear	
G A R	10.	**NBC/Toxic Industrial Materials**	
G A R	a.	-NBC threat	
G A R	b.	-TIMs identified by intelligence sources[1, 2]	
G A R	c.	-local industrial facilities identified[1, 2]	

TABLE 2. ENVIRONMENTAL HEALTH SURVEILLANCE

A. General Setting **POC:** **Phone/Email:**

Status	No.	Required Elements	Field Notes (use reverse if needed)
G A R	1.	Is base camp drainage adequate?	
G A R	2.	Are living/work facilities adequate? What Type?	
G A R	3.	How many personnel are living in each facility?	
G A R	4.	Are hand washing facilities provided throughout base camp?	
G A R	5.	Are there adequate showers provided for personal hygiene?	
G A R	6.	Is there an adequate number of latrines provided?	
G A R	7.	Is human waste disposed in an environmentally sound manner and in a manner that protects human health?	
G A R	8.	Are laundry facilities/services provided or planned?	
G A R	9.	What diseases are endemic to this area? Have personnel received a medical threat briefing? By whom? When?	
G A R	10.	Was the site used for any previous industrial/agricultural activity? If yes, describe.	
G A R	10a	Is there a history of pesticide applications on the site? If so, document as well as possible to establish baseline exposure information.	
G A R	11.	Are existing buildings to be occupied by US forces? If so, which ones? What have the buildings been used for previously?	
G A R	12.	Are any nearby receiving bodies of water to be used for wastewater disposal?	
G A R	13.	Are hazardous materials to be stored in a manner that is environmentally sound and will protect human health?	

TABLE 2. ENVIRONMENTAL HEALTH SURVEILLANCE

A. General Setting **POC:** **Phone/Email:**

Status	No.	Required Elements	Field Notes (use reverse if needed)
G A R	14.	Are there any pools of water or other liquids of unknown quality?	
G A R	15.	Are there any locations where wastes have been disposed of incorrectly and may cause a hazard to soldiers?	
G A R	16.	Is solid waste (to include regulated medical waste) disposed in an environmentally sound manner and in a manner, which protects human health?	

TABLE 2. ENVIRONMENTAL HEALTH SURVEILLANCE

B. Food **POC:** **Phone/Email:**

Status	No.	Required Elements	Field Notes (use reverse if needed)
G A R	1.	What facilities are used for food service (MKT, temper tent, etc?)	
G A R	2.	What types of rations are being consumed (As, Ts, MREs, UGRAs)?	
G A R	3.	Is all food served in dining facilities DoD approved?	
G A R	4.	What is the source of the food?	
G A R	5.	Do the supporting vets perform routine wholesale inspections?	
G A R	6.	Are food handlers food sanitation trained? What language do they speak?	
G A R	7.	Are adequate food storage facilities provided?	
G A R	8.	Are host nation eating establishments off limits?	
G A R	9.	Have all food service personnel been medically screened?	
G A R	10.	How often are food service facilities inspected?	
G A R	11.	How is liquid kitchen waste disposed? Is it in a manner that is environmentally sound and protects human health?	

TABLE 3. ENTOMOLOGY

Status	No.	Required Elements	Field Notes (use reverse if needed)
A. Individual Protective Measures		**POC:**	**Phone/Email:**
G A R	1.	Arthropod repellent on-hand and being used?	
G A R	2.	Uniforms properly worn? (pants tucked in, sleeves down)	
G A R	3.	Uniforms treated with Permethrin? Permethrin on-hand?	
G A R	4.	Bed nets on-hand and being used?	
G A R	5.	Do soldiers have *Tick & Rodent Cards*?	
G A R	6.	Do soldiers/medics have tick removal forceps?	
G A R	7.	Buddy system checks for ticks?	
G A R	8.	"*Staying Health in ?*" Guides distributed?	
G A R	9.	Individuals practicing good personal/unit area sanitation?	
G A R	10.	Education on avoidance of pests and pest habitats?	
B. Arthropod/Rodent Surveillance		**POC:**	**Phone/Email:**
G A R	1.	Surveillance program in place?	
G A R	2.	Larval mosquito surveillance (dip sampling)	
G A R	3.	Adult mosquito surveillance (light traps, landing collections, etc)	
G A R	4.	Biting flies surveillance (landing collections)	
G A R	5.	Filth flies (fly sticky strips, baited fly trap)	
G A R	6.	Fleas and lice (complaints; sick call records; flea trap)	
G A R	7.	Cockroaches (visual and trap surveillance)	
G A R	8.	Ticks and mites (Skin rashes, tick drags, complaints)?	
G A R	9.	Stored product pests (rations infested/destroyed)?	
G A R	10.	Venomous Arthropods (scorpions, centipedes, ants, etc. collected or seen)	
G A R	11.	Commensal rodents (traps, glue boards, droppings, burrows)?	

TABLE 3. ENTOMOLOGY

Status	No.	Required Elements	Field Notes (use reverse if needed)
G A R	12.	Venomous animals (poisonous snakes collected or seen)?	
G A R	13.	Is the Health Clinic/Surgeon aware of arthropod threats?	
G A R	14.	Health Clinic/Surgeon and entomologist communicating?	

TABLE 3. ENTOMOLOGY

C. Arthropod/Rodent Control **POC:** **Phone/Email:**

Status	No	Required Elements	Field Notes (use reverse if needed)
G A R	1.	Control program for disease vectors?	
G A R	2.	Installation Pest Management Program?	
G A R	3.	Integrated Pest Management procedures used?	
G A R	4.	Reduction of food sources and breeding habitats?	
G A R	5.	Control personnel properly trained and certified?	
G A R	6.	Medical surveillance of pest control personnel?	
G A R	7.	Respiratory protection program for pest control personnel?	
G A R	8.	Personal protective equipment for pest control personnel?	
G A R	9.	List of pesticides currently used	
G A R	10.	Pesticide labels and MSDSs on hand? Bilingual labels for contractors?	
G A R	11.	Are pesticides EPA and DoD approved?	
G A R	12	Are “local” pesticides being used. If so, have they been approved for use by the appropriate medical authority or pest management professional?	
G A R	13.	Is all pesticide usage recorded on DD Form 1532-1, or equivalent?	
G A R	14.	Is DD Form 1532-1 information forwarded monthly to USACHPPM for archiving?	
G A R	15.	Are pesticides stored in proper facility?	
G A R	16.	Is an eyewash kit available?	
G A R	17.	Is spill control equipment/supplies on hand?	
G A R	18	Is the proper pest control equipment available and maintained properly?	
G A R	19.	Are bilingual pesticide-warning signs posted at storage facility?	

TABLE 3. ENTOMOLOGY

C. Arthropod/Rodent Control **POC:** **Phone/Email:**

Status	No	Required Elements	Field Notes (use reverse if needed)
G A R	20.	Is pesticide application equipment calibrated and working properly?	
G A R	21.	Personal information distributed to soldiers (tick & rodent cards, staying healthy guides)?	
G A R	22.	Are soldiers aware of and conducting tick checks?	
G A R	23.	Is *Contingency Pest Management Pocket Guide* available?	
G A R	24.	Are Unit Field Sanitation Teams manned, trained, and equipped?	
G A R	25.	Current threat assessment information on hand (AFMIC CD-ROM, Disease Vector Ecology Profile, Vector Risk Assessment Profile, etc.)?	
G A R	26.	Have action thresholds based on the medical threat been developed to guide initiation of pest control operations? (Incorporate values into Tables following?)	
G A R	27.	Contracts: Are pest management contracts written IAW TIM 39 guidance and reviewed prior to award of contracts?	
G A R	28.	Contracts: Are pest management contracts overseen by a qualified QAE?	

[1] ***NOTE: This may be classified information***
[2] ***NOTE: This may be NATO Restricted Information***

CHAPTER 3: Introduction to Arthropod Sampling

1. Background.

Surveillance programs depend upon sound sampling methodology, and on reliable methods of identifying vectors. Arthropod sampling is the most important entomological activity associated with the surveillance of vector-borne diseases. Sampling arthropod and other animal pest populations is also an important and integral part of IPM and Force Health Protection (FHP). Quantitative, objective sampling will lead to inferences about population dynamics and disease threat assessments which will play an important role in developing disease counter-measure recommendations to commanders and medical personnel. If the number of infective bites is reduced by command-emphasized counter-measures, then the cases of vector-borne disease in military personnel will be prevented or greatly reduced.

Field assessment of vector-borne diseases involve, to a large extent, study of the vector and its ecology, particularly vector densities. Accurate density estimates are essential to understanding the dynamics of vector populations over time and making correct threat assessments from this information. Choosing the appropriate sampling method is an important consideration in vector arthropod and animal sampling because this will determine the usefulness of the resulting FHP estimates and recommendations. The choice of the sampling method may affect the precision, accuracy, and fidelity of population estimates. The adequacy of the information provided by a sampling scheme is defined by the precision (small variance) and accuracy (lack of bias) of sample estimates. **Precision** is the degree of statistical error associated with an estimate and can be affected by the sample size. The **accuracy** of the estimate is reflected by how close that estimate is to the true value. Accuracy may not always be relevant for assessing relative estimates of abundance because these may be measured in units that are unrelated to absolute density or have some systematic deviation from the true value. In this case, **fidelity**, or the ability of a relative measure of abundance to reflect real population changes over time, may be a more relevant measure of the sampling reliability.

Although not the major emphasis of this guide, the operational medical entomologist must be aware of attempts by the medical community to diagnose disease in sick military personnel and to survey the health of the surrounding civilian population. Diagnosis of sick patients in a military hospital is usually easily accessible to the medical entomologist, who only has to ask to see the results of laboratory tests in the Dept. of Pathology or poll physicians who are treating patients with infectious diseases. The surrounding civilian population's experience with vector-borne diseases may be more difficult to determine. The information may be contained in publications, in unpublished reports of local public health authorities, or in the results of active surveillance (i.e., where teams sample the population before individuals seek medical attention)

performed by epidemiology teams. Often, active surveillance of a healthy population is performed by taking blood samples from many individuals and then testing the samples for presence of antibodies to specific pathogens (sero survey). Sero surveys are plagued with inaccuracies (lack of sensitivity or the ability to detect people actually exposed to the pathogen; or lack of specificity or the ability to be sure that a negative result means a lack of exposure), but they give the fastest and most realistic appraisal of the presence of vector-borne disease because each person is exposed to many vectors and antibodies typically persist for weeks or months.

One of the most common uses of arthropod sampling data in surveillance is the estimation of vector density. This technique is based on the concept (though not always true) that high vector densities are associated with outbreaks of vector-borne diseases. The correlation between disease cases in human or animal hosts and estimated vector density often is difficult to demonstrate and will vary according to a number of factors. There are two major categories of population estimates: subjective and objective. Subjective estimates (also known as qualitative surveys) imply frequency of occurrence and use terms such as “rare”, “common” or “abundant”. However, these terms are vague and of little use in describing the entomological and vector-borne disease threat. Qualitative surveys are commonly used for inspection of commodities or during retrograde cargo inspections and are often used for newly established pests. Objective estimates (numbers/unit area), such as absolute estimates (=direct counts), are very time consuming, expensive and unrealistic in that it is impossible to count every arthropod in a population. **Relative estimates of populations** are best suited to military deployment situations. These estimates are typically related to sampling method (e.g., # of flies/ sweep net sample) and are much less time consuming than absolute estimates. However, these estimates are influenced by changes in efficiency of sampling technique.

Determining if a sample is representative of the total population requires that the sample size be adequate and that there not be any biases when the samples are taken. For a sample to be unbiased it must be taken at random. **Random sampling** implies that each measurement in the population has an equal opportunity of being selected as part of the sample. Since the aim is to give every unit of the population an equal and independent likelihood of being chosen, the basic approach to randomizing samples is to let chance determine the samples. Random decisions can be made by drawing numbers from hats, flipping coins, rolling dice, etc. However, animals in nature are usually not randomly distributed, but rather are aggregated in favorable habitat areas. For example, light traps for collecting adult mosquitoes usually collect more mosquito adults if they are placed in shaded areas near vegetation and protected from the wind. Contrasted with random samples are **systematic samples**, which involve taking samples that have some sort of systematic or regular arrangement (e.g., larval mosquito dip samples taken every 10 feet**). Systematic sampling is by far the easiest way to obtain a sample from a**

large population. Here one picks samples according to some predetermined rules; say one dip for larval mosquitoes every five steps in a row. However, care must be exercised not to introduce subjectivity (bias) into the choice of samples. Most of the bias can be removed by setting the rules prior to seeing the "sampling universe" so that the location of the samples relative to the distribution of the population is unknown. **Serial sampling** consists of repeating the same sampling procedure at several or many different points in time with the objective of measuring changes in arthropod numbers. For example, adult mosquitoes are usually sampled at the same locations throughout the mosquito season. "**Presence-absence**" sampling is a quick and easy way to obtain information on the proportion of occupied (or unoccupied) sample units. However, this sampling procedure provides no information concerning the quantitative population estimate and may be of little value in determining the vector-borne disease threat.

The number and size of samples are important in relation to the accuracy and precision of the estimates obtained from sampling data. In conducting an entomological assessment, the maximum amount of information should be obtained for the minimum amount of effort. Because sampling is generally labor intensive and time consuming, an individual must consider such things as location, size, and number of samples required. In general, a large number of small- or medium-sized samples are better than a small number of large-sized samples, since the former gives a better picture of the variation within the population being sampled. On the other hand taking more samples than is necessary for a particular problem is a waste of time and resources. A simple, homogenous area will require less sampling than a complex, heterogeneous one. Dispersion of the population to be sampled must be considered. **Dispersion** refers to the way in which individuals within a population are arranged in space, relative to one another. This pattern of spacing can range along a continuum from being completely uniform (all individuals are evenly spaced) to being extremely clumped (all individuals are at the same location).

Sampling is a procedure whereby a property of a population (the total, true or absolute population) is determined by examining a relatively small proportion of the true population. The **sample** is the proportion of the population that is actually examined. In order to reflect the property of the true population, the sample must accurately reflect or represent the variability or variation in the true population. The sample is then a **representative sample**. The problem, of course, is how many observations (=samples) should be made in order to obtain a representative sample of the true population. This problem involves the determination of **optimum sample size**. An associated problem is deciding on the unit of observation or a unit of area of habitat (e.g., a hectare of land or cubic meter of water). The problem is to determine that **optimal sample unit**.

Selection of **sample unit** by logical habitat structure is extremely important in entomological assessments. For example, it seems logical to select the surface

of water pools among aquatic vegetation and floating debris for sampling *Anopheles* mosquito larvae, as this represents their preferred larval habitat. Selection of an appropriate **sample unit size** is also important. Ideally the physical size of the sample unit should be large enough so that the probability of obtaining a zero count is minimal. An example of a sampling unit is the number of ticks per area covered by a tick drag, which is then converted to number of ticks per hectare or acre.

Relative population estimates (the number of organisms caught in nets or traps but which cannot be related to area in any way) are often used and can provide valuable qualitative information about presence or absence and abundance. **Population density** (the number of organisms per unit area) can provide valuable quantitative population estimates. **Absolute density** (all individuals in a unit area) or the exact numbers of arthropods is not practical to obtain. However, **relative density** (numbers of arthropods related to other numbers in time or space) can be used for comparisons and is often related to the sampling method used.

Repeated sampling of a population can yield information on rates of population change within an area and the resultant effects on FHP. However, repeated sampling is commonly not possible during deployed situations, particularly during combat or high threat situations. In this case, FHP assessments and recommendations may need to be made with little available data. This is where previous field (i.e., deployment)) experience and a thorough knowledge of vector ecology will greatly enhance the FHP assessment process.

Ideally, any index of a biting arthropod population can be translated to some sort of estimate of the number of bites likely to be received by an individual in a given period of time. An estimate of the bites received combined with the infection rate of the vector can produce a very realistic estimate of disease risk. When available, such estimates are highly motivating to commanders who can deploy the resources necessary to achieve effective vector control. Unfortunately, the efficiency of any trapping technique is likely to vary with the density of the population in a relationship that is not the same as that for human biting collections. In other words, collecting twice as many mosquitoes in a light trap does not mean that a human will likely receive exactly twice as many bites in the same area. Because of this difficulty of relating trapping results to estimations of biting pressure on humans, it is usually most accurate to establish a treatment threshold for the trapping results. This threshold is usually a conservative estimate of the number of vectors collected in a trap that indicate a significant threat of disease transmission. This threshold has been estimated for a very few arthropod-borne diseases (e.g., dengue transmission is considered unlikely when an average of less than one *Aedes aegypti* vector bites per hour). In most situations, the practicing entomologist may have to start with a very rough estimate of the threshold, which he/she later refines by comparing his own results in the area of operations. Over a relatively short period, the

entomologist will be able to prioritize application of vector control based on trapping results. If the disease is sufficiently well monitored, the entomologist may even be able to use trapping results to estimate disease risk with the area of operations.

2. Arthropod Sampling Techniques.

a. Introduction.

Many sampling tools are available for arthropods. The choice of which tool to use will depend on the arthropod sought, the specific objectives of the surveillance program, and the time and resources available. If the isolation of pathogens from arthropod specimens is desired, methods must be used to prevent deterioration of the specimens before they can be tested for pathogens in the laboratory.

b. *In situ* (Latin = in place) sampling.

In place sampling is typically used for large and conspicuous insects viewed in the habitat. This is usually done when the numbers of insects are low and the plant cover is sparse. Sometimes when the numbers of arthropods are high, a frame (usually 1 square meter) is used and all the arthropods within the frame are counted.

c. Catch per unit effort.

This technique involves counting arthropods collected or over a fixed time or area. This can be accomplished by using line transects or nets (especially sweep nets) or insect vacuums. Transects or observations along a set path and set time can provide for site-to-site comparisons between locations using the standardized formula: P = 100NA/L, where P = population index, L = length of transect in meters, A = size of dispersal area in hectares, and N = arthropod numbers per 100 meter of transect. This technique can be labor intensive and time consuming. It also exposes the collector to potential environmental dangers such as chemical contaminants of the area, land mines, etc.

d. Trapping.

Trapping techniques exploit some behavioral characteristic of the target arthropod. Interception traps for air (e.g., Malaise, Manitoba, window) or soil (pit-fall) exploit the dispersal (either flying or crawling) behavior of certain arthropods. These traps simply collect arthropods that either fly or crawl into them during their normal locomotor/dispersal activities. Attraction traps such as light traps (white light or black light (UV)) or baited traps (e.g., CO_2 traps for

mosquitoes, pheromone traps, and attractant (octenol) traps for mosquitoes) are less time consuming and minimize environmental exposures of the collector.

e. Indirect techniques.

Estimating population density by measuring insect effects or products such as frass collection for termites are helpful in specific situations.

3. Sampling Parameters (how and what to sample)

a. Sample Size (number of samples).

In the planning of a sample survey, a stage is always reached at which a decision must be made about the size of the sample. This decision is important. Too large a sample implies a waste of resources, and too small a sample diminishes the value and utility of the results. The total number of samples depends on the degree of precision required. When estimating population density, the usual criterion is that the estimate of mean density should be within 20% of the true value (standard error of 0.2 or 20% of the mean). More precisely, this criterion should read that we want to be 95% sure that the estimate is within 20% of the true value. The decision cannot always be made satisfactorily; often you will not possess enough information to be sure that your choice of sample size is the best one. However, sampling theory provides a framework with which to think intelligently about the problem and make sound decisions.

The required number of samples (*n)* can be roughly estimated by statistical methods, but these methods are not easy to use and require statistical computations as described below.

$$n = \left(\frac{s}{E(\mathrm{x})} \right)^2$$

Where *s* = standard deviation, *x* = mean and *E* is the predetermined standard error as a decimal of the mean (i.e., normally 0.20, if you want to estimate of mean density within 20% of the true value). This expression compares the standard deviation (s) of the observations with the standard error (*E*) acceptable for the contrasts you need to make. From this equation in any given situation the value of the standard error will change with the square root of the number of samples: thus a large increase in *n* is necessary to bring out a small improvement of *s*. Since the number of samples required depends on the mean (x) and standard error *E*, it becomes necessary to know these values *a priori*. Although this is obviously impossible, we often have an idea of what to expect.

An example of how to calculate sample size (*n*) for mosquito light trap collections is as follows. A value of 0.2 is chosen for *E,* providing an estimate of mean density within 20% of the true (actual) value. A preliminary collection has indicated that a mean (*x*) of 10 mosquitoes are collected per light trap. The individual traps counts ranged from 6-16 mosquitoes per light trap, thus yielding a standard deviation (*s*) of approximately 4. Using the formula above, *n* is calculated to be 4. This means that 4 light traps are needed if you want to be sure that you data be within 20% of the actual value. Conversely, you would need 64 light traps to ensure that your data is within 5% (*P* = 0.05) of the actual value. This illustrates that indeed a large increase in *n* is necessary to bring out a small improvement of *s*.

Population density is always changing, so no one can ever know precisely what value of *s* to use in the above equation. Furthermore, sampling is not an end in itself, but, rather, a means of acquiring information for some subsequent use. Emphasis should be placed on drawing correct conclusions from the sampling data, while selecting sample size should be of secondary importance. Sample size is usually a compromise between the need for accuracy and the cost (and time) of obtaining the data. Sampling can be very time consuming and thus expensive. To run an efficient program, one has to develop sampling schemes that get enough information with as little time as possible. **A sampling rule of thumb is: take at least five samples; never take less than 3 samples**.

b. Time to sample.

Timing of sampling can be crucial and depends on the occurrence of the pest, its lifecycle, and the stage being sampled. Typically the timing of the sampling needs to coincide with the peak numbers of a particular stage. Remember life is not black and white – there will be individuals in a population that represent several different ages. For example, if the duration of the first instar lasts five days for a particular insect. When you sample that stage there will be individuals that are zero, one, two, three, four, and five days old in that instar. What you are shooting for is having the majority of individuals around the three-day point. The spread of numbers around that point will look like a bell-shaped curve or “normal” distribution.

c. Arthropod stage.

Because arthropods exhibit metamorphosis, that stage sampled can be an important consideration. The damaging (or disease carrying) stage is often most important and often the one that is sampled. For disease-vectors, this generally means the adult stage because it’s the adults that transmit diseases. If substantial lead-time is needed to affect a particular management strategy, then earlier stages (e.g., larvae) may need to be sampled to gain enough information to make accurate predictions.

d. Pattern of sampling.

The spatial sampling pattern will depend on the pest species, disease threat, population dispersion, season of the year, enemy threat, and time and resources available. As previously discussed, systematic sampling is recommended whereby predetermined rules are used with a rudimentary knowledge of the arthropod pests in the area.

4. Action Threshold (AT)

a. The decision to use an insecticide, or take some other action, against an insect infestation requires an understanding of the level of disease, damage or insect infestation that can be tolerated without an unacceptable economic or health loss. The level of infestation or damage at which some action must be taken to prevent an economic or health loss is referred to as the action threshold. The concept of action thresholds is the backbone of any sound integrated pest management program. However, entomologists have been slow in establishing action thresholds for medically important arthropods. The reason is probably related to difficulties in making these determinations. In general, action thresholds are not fixed in time or space for any given pest or situation. Usually the threshold varies from region to region, from year to year, and with the vector-borne disease situation. The basic need to determine an action threshold of a pest is to distinguish between its mere presence in an area as opposed to the population density that will cause unacceptable quality of life, morbidity and mortality.

b. The AT is a practical or operational rule, rather than a theoretical one. Action threshold is further defined as "the population density at which control action should be determined (initiated) to prevent an increasing pest population (injury) from reaching the economic injury level." Although measured in insect density, the AT is actually a time to take action, i.e., numbers are simply an index of that time.

Action Threshold:

- the most widely used index in making IPM decisions
- the number of insects when management action should be taken
- also called Economic Threshold
- an index for WHEN to implement control activities
- intent is to prevent insect populations from exceeding the injury level.

The AT is a useful concept because it quantifies the cost/benefit ratio that underlies all pest control decisions. In practice, however, it is not always necessary or desirable to wait until a population reaches injury level before initiating control operations. Once it is determined that a population will reach outbreak status, prompt action can maximize the return on a control investment. Since there is usually a lag time between the implementation of a control

strategy and its effect on the pest population, it is always desirable to begin control operations before the pest actually reaches the injury level.

c. To quantitatively compute action thresholds one needs a series of paired points that allow regression of the disease increase with population levels. Disease rates must be known to detect some measurable increase of disease with various levels of infestation. However, models of the dynamics of a vector population and its interactions with the pathogen and vertebrate host are generally more complex and have larger numbers of variables than models of pest-crop systems. The objectives of a sound pest-management system are to "ensure favorable economic, ecological, and sociological consequences". These objectives become more difficult to determine quantitatively as we attempt to weight the effects of human disease on a communal, national, or global basis.

5. Purpose of Arthropod Surveillance.

a. Determine the presence of pest and vector species.

b. Determine if vector species are infected with certain pathogens.

c. Monitor population numbers to determine…

(1) when thresholds are passed.

(2) when to initiate control measures.

d. Estimate the effectiveness of control measures.

6. Principal Steps In A Sample Survey.

This section briefly describes the steps involved in the planning and execution of an entomological survey.

a. Objectives of the survey.
Determine the purpose of the survey?

b. Population to be sampled.
The population to be sampled (the *sampled* population) should coincide with the population about which information is wanted (the *target* population).

c. Data to be collected.
Verify that all data are relevant to the purposes of the survey and that no essential data are omitted.

d. Degree of precision needed.

The results of sample surveys are always subject to some uncertainty because only part of the population has been measured and because of errors in measurement. This uncertainty can be reduced by taking larger samples and by using superior instruments of measurement. But this usually costs increased resources. Consequently, the specification of the degree of precision wanted in the results is an important step.

e. Methods of measurement.

There may be a choice of measuring instrument and of method of approach to the population.

f. Sample units.

The construction of the list of sampling units is often one of the major practical problems. These units must cover the whole of the population. Sometimes the appropriate unit is obvious, such as mosquito larval sampling in a single tire. Sometimes there is a choice of units, such as determining which of many temporary pools to sample for mosquito larvae.

g. Sample size.

For each plan that is considered, rough estimates of the size of samples can be made from knowledge of the degree of precision desired. The relative time and other resources available for each plan are also compared before making a decision.

h. Pretest.

It may be very useful to first try out the field methods on a small scale. This nearly always results in improvements in the methods and may reveal other troubles that will be serious on a large scale.

i. Organization of fieldwork.

In extensive surveys many administrative problems may be encountered. Personnel may require training in the purpose of the survey and in the specific methods of measurement to be employed and must be adequately supervised in their work. A procedure in e*arly* checking of the quality of the returns is invaluable.

j. Summary and analysis of the data.

The first step is to edit the completed data sheets, in the hope of amending recording errors, or at least of deleting data that are obviously erroneous. Thereafter, the tabulations that lead to the estimates are performed.

k. Information gained in future surveys.

The more information you have initially about a population, the easier it is to devise a sample that will give accurate estimates. Any completed survey is potentially a guide to improved future surveys, in the data that it supplies (e.g. means and trends), the nature of the variability, and about resource costs involved in getting the data.

CHAPTER 4: Mosquitoes (Family Culicidae)

1. Military Importance.

Mosquitoes rank first of all the arthropods that impact the health of humans and military personnel during deployments. Not only do they transmit disease, but also they occur in such numbers that they cause great annoyance. Three genera are of particular medical concern, *Anopheles, Aedes*, and *Culex*. Only mosquitoes of the genus Anopheles transmit human malaria. Dengue is only transmitted by the genus *Aedes*. The common yellow fever mosquito, Aedes aegypti, transmits yellow fever from human to human. *Culex* mosquitoes transmit certain filarial worms that produced the disease known as filariasis. In addition, mosquitoes transmit many human encephalitides caused by viruses (West Nile, Japanese, equine, St. Louis). Pest mosquitoes degrade mission readiness and combat effectiveness through severe annoyance, itching bites, loss of sleep, and nervousness.

2. Background.

Mosquitoes are two-winged insects belonging to the order Diptera, family Culicidae. Mosquitoes are probably the most important arthropod pest species encountered by preventive medicine personnel. Mosquitoes are the sole vectors of pathogens causing many serious diseases such as human malaria (the leading cause of disability and death worldwide), Chikungunya virus disease, and dengue, and they are of prime importance in filariasis (WHO ranks lymphatic filariasis as the second leading cause of disability worldwide), and viral encephalitides of humans. The danger of these diseases is present in many tropical and sub-tropical areas worldwide. The danger is particularly acute for American military personnel because they typically have no immunity to these diseases.

The annoyance factor to humans caused by large mosquito populations is an equally important factor and cannot be overstated. Biting mosquitoes can make training and mission accomplishment difficult and generally degrade morale and productivity of military personnel. Mosquito annoyance alone, independent of the disease potential, is often used to justify control efforts.

In general, mosquitoes are not exceptionally strong fliers, and most species probably migrate no more than 4-5 kilometers from their breeding areas. However, migrations of black salt marsh mosquitoes (*Ochlerotatus taeniorhynchus*) into populated areas from a distance of over 90 kilometers have been documented. In contrast, adult *Aedes aegypti* mosquitoes (vectors of dengue and yellow fever) only disperse 100 meters or less (roughly one city block). Only female mosquitoes pierce the skin of animals and feed on blood, the protein from blood being required for eggs to develop. . Females with ample

food may live 4 or 5 months, particularly under conditions of hibernation. Male mosquitoes usually remain alive for no longer than 3 weeks.

3. Geographic distribution and habitat.

Mosquitoes have a worldwide distribution and can occur at elevations as high as 4,300 meters and below sea level in mines. They are abundant in the tropics, and almost unbelievably large swarms of them occur in the Artic. Therefore, preventive medicine personnel should always conduct routine mosquito surveillance (except in extreme desert climates) when the average daily temperature is above 50^0 F. All mosquito larvae are aquatic and feed on microorganisms and other particulates in the water. Mosquitoes lay their eggs either singly on the surface of the water (i.e., *Anopheles*) or in rafts (i.e., *Culex* and *Culiseta*). *Aedes and Ochlerotatus* species lay their eggs in containers or on moist dirt or mud that is subject to inundation by tidal water, seepage, overflow, or rainwater. One feature that all such sites have in common is protection of the ovipositing female from action of wind and wave. The larvae of most culicine species hang suspended diagonally from the water surface by means of a prominent breathing siphon. In contrast, the larvae of anopheline species lie suspended horizontally just beneath the surface of the water, by means of palmate hairs. Sight identification of genera of larval mosquitoes is an extremely useful skill for the operational entomologist. Armed with this knowledge, the entomologist can begin forming opinions about the vector population before returning to the laboratory and while the landscape is still in full view. In addition to the position of the larva in the water, the swimming motions of the larvae are also characteristic of groups of genera. *Anopheles* larvae move very quickly in a "kick and glide" motion, skittering just below the surface tension film. *Aedes* larvae move their bodies symmetrically during a swimming motion, creating the impression of a series of "S" shapes to the eye. Some species move more quickly than others, but they all form an "S." *Culex* larvae do not move their bodies as symmetrically, so that their movement appears more erratic as they move through the water column. Only female mosquitoes pierce the skin of animals and feed on blood, the protein from blood being required for eggs to develop. A useful generalization about female mosquito longevity is that about 10% per day die under good conditions and 30% per day die under less favorable conditions. One of the challenges for effective vector control is to decrease the rate of survival so that the female mosquito does not have time to develop an infective dose of a pathogen it has picked up in a previous blood meal.

4. Procedures.

Good maps are essential in planning and conducting mosquito surveillance. They are used for orientation and location of larval breeding sites and adult sampling sites in relation to the living and working areas to be protected. Small-

scale topographic maps should be used to plot all collection sites and potential larval breeding areas.

a. Mosquito Egg Surveillance

(1) Background. Mosquito eggs are found in a variety of different habitats (e.g., small pools, marshes, rock pools, tree-holes, plant axils, flower bracts, fallen leaves, bromeliads, and a wide variety of man-made (=artificial) containers. Some species lay their eggs singly, while others lay them in egg rafts or in sticky masses glued to the under sides of floating debris. Many species deposit their eggs directly on the surface of the water, however a large number oviposit not on the water surface but a short distance from the water's edge among leaf litter, mud and debris or on the walls of man-made containers, tree-holes, or on plants.

(2) Purpose. The purpose of mosquito egg collecting is to determine the presence and abundance of artificial container-breeding mosquitoes (i.e., *Aedes aegypti* and *Ae. albopictus*). Egg collections using ovitraps are a very effective means of monitoring populations of these mosquito species. This form of sampling is particularly important in areas of the world were dengue fever is found. Because of the great diversity of oviposition sites various sampling techniques would be required if eggs from many different mosquito species were to be adequately sampled. Few methods for sampling mosquito eggs have been developed and most that have been developed require specialized equipment and tend to be labor intensive (e.g., Horsfall's soil washing machine and various other egg extraction methods).

(3) Procedures. Container-breeding mosquito ovitraps are a sensitive and efficient technique for detecting populations of container-breeding mosquitoes (primarily *Aedes aegypti* and *Aedes albopictus*). Many vector biology and public health workers in North America have used ovitraps as a routine surveillance method. One mosquito inspector can cover 3 to 5 times more area using ovitrap surveys compared to larval surveys. It has been shown that ovitraps are more sensitive (up to 40% more sensitive) than larval surveys in detecting the presence of *Ae. aegypti* and *Ae. albopictus*. This survey technique is an essential prerequisite for the planning and execution of management strategies for dengue vector control.

(a) Oviposition trap. This trap consists of a black plastic cup with tapered sides and having a capacity of about 1 pint (approximately 3 inches in diameter and 5 inches high). Oviposition traps can be made by painting a one-pint capacity jar glossy black on the outside. A 1-inch wide, 5-inch long red, velour paddle is attached (rough side towards inside of cup) with a paper clip to the inside (center) of the jar. If velour paper is not available, make egg collection strips out brown or white colored heavy-duty paper towel. Eggs of Aedes spp. are deposited just above the water line on the rough side of the paddle.

(b) Number of traps. Set up 10 collection sites using a **minimum of 5 oviposition traps at each site** (50 traps total). Include disposal yards and areas where tires are stored as well as residential areas.

(c) Trap placement. Normally, only one ovitrap is placed at each collection site. Place the oviposition traps where they will be in full or partial shade most of the day.

i. Near walls, fences, hedges, shrubs, used tires, piles of junk, or other sheltered areas that are potential resting sites and protected from the sun and wind.

ii. Near or at ground level with at least 12 inches or open space above the ovitrap.

iii. Where they are inconspicuous to children or animals, yet can be easily reached to be serviced.

iv. DO NOT place oviposition traps near garden and lawn sprinklers or where rain run-off might fill or flood the ovitrap. DO NOT place oviposition traps in direct sunlight, windy or fully exposed areas.

(d) Trap setup. Add 1 to 2 inches of water to each ovitrap. Do not use chlorinated water. Clip or staple the egg collection strip with the rough surface of the strip facing the center of the container. Label the egg collection strip with identifying information, in pencil, on the smooth side before clipping in the trap. Tip the container to wet the strip before placing the container at its site.

(e) Trap inspection.

i. Inspect oviposition traps at least weekly (eggs may hatch if a longer collection cycle is used) and inspect all ovitraps on the same day. Some mosquitoes, which are not being surveyed by this method (i.e., *Culex*), will lay eggs in rafts on the surface of the water. These eggs may hatch in 2 or 3 days. Collect the paddles weekly

ii. Eggs are usually deposited just above the water line on the exposed rough side of the strip.

iii. Place collected strips in an insulated box that is lined with dry paper towels or in self-sealing plastic bags to return to the laboratory. Be sure to protect the rough or egg side of the strips to avoid dislodging or crushing the eggs.

iv. Clean the oviposition trap inside and out to maintain a smooth shiny appearance each time egg strips are collected.

b. Mosquito Larval Surveillance.

(1) Background. Mosquito larvae occur in various types of aquatic habitats, varying from lakes and marshes to small temporary pools and collections of water in tree holes, leaf axils of plants and artificial containers. Larval collections are an important part of overall mosquito surveillance. Larval collections are used to determine requirements for control operations and to detect the presence of important species that are not attracted to light traps as adults, such as certain species of *Anopheles, Ae. aegypti and Ae. albopictus*. **Control efforts based on larval populations are preferred** because significant populations can be eliminated by chemical control, predation, parasitism and natural mortality before the mosquitoes become adults. In addition, pesticide applications can be pinpointed to only those areas where mosquito populations are known to exist, thus minimizing environmental hazards. Once mosquitoes become adults they become more difficult to control because their dispersal requires a greater area to be treated and it becomes more difficult to get the pesticide to areas where adult mosquitoes seek shelter, such as vegetation and buildings.

(2) Purpose. Larval mosquito surveillance is used to precisely determine the exact areas in which mosquitoes breed and their relative abundance. For these reasons, larval mosquito surveillance is of particular value in guiding larval control operations.

(3) Procedures.

(a) During the **initial larval survey** every conceivable aquatic habitat within a 2-mile radius of the area to be protected should be considered as a potential mosquito-breeding site. Generally, the majority of species will not move more than 2 miles in great numbers (exceptions include *Ae. vexans* and *Ae. sollicitans* that are apparently able to travel tens of miles on their own power and even further when they get caught in weather systems). However, since the number of mosquitoes traveling greater distances tends to decrease, surveys even one mile from the area to be protected are useful. Some species like *Ae. aegypti* and *albopictus* usually travel less than 100 m. Detailed topographical maps are needed to locate all of the large bodies of water including natural and artificial lakes, ponds, streams, and reservoirs. In a rural area, up-to-date topographical maps usually will even show the location of farm yards in which many breeding sites will be found upon field inspection. In urban areas, they will pinpoint highways, transmission and railroad rights-of-way and industrial parks. In any case, they are an excellent aid for both larval surveys and actual spray operations. Mosquito larvae are usually found where surface vegetation or debris is found. Larvae are generally more abundant in shaded areas, near vegetation or at the water's edge. It is necessary to proceed slowly and

carefully when searching for mosquito larvae, since disturbance of the water or casting shadows may cause the larvae to dive to the bottom. Because larvae tend to "bunch up" rather than being uniformly distributed, it is often necessary to sample several parts of a water body. Each site should be sampled frequently during the breeding season.

(b) For **routine larval surveillance**, the number of larval collection sites depends on the number of breeding sites identified within a 2-mile radius of the area to be protected. A representative number (10 to 20 percent – three stations minimum) of the breeding sites identified during the initial larval survey should be selected for weekly collections. Also, periodically check for breeding, especially following heavy rains, other locations positive for mosquito breeding in the initial survey.

(c) Monitor larval sites for mosquito breeding at least once a week during the breeding season.

(d) The primary collection method for larval mosquitoes is **dipping**, using a white enameled or plastic dipper. A large squeeze bulb syringe, approximately 50 ml (commonly called a “turkey baster”) can be inserted into tree-holes and tires to remove the water that contains larvae.

(e) When dipping for culicine species, employ a quick, intercepting movement in the water while twisting the wrist to fill the dipper with minimum disturbance of the water, since larvae quickly swim away, dive, or hide. The collector’s shadow will disturb the larvae as they move across the water so be cautious not to approach the sampling site with the sun at your back.

(f) To dip for anopheline mosquitoes, skim the dipper along the surface of the water in places where vegetation or floating debris offers protection for the larvae. Be careful not to skim for so long a stretch that the water flushes captured larvae from the dipper. Alternatively, place the dipper near emergent vegetation and slowly lower the dipper into the water allowing the surface water to rush in and carry the larvae into the dipper.

(g) Conduct sampling by evenly distributing the dips taken over the breeding site in order to obtain a representative sample of the mosquito population.

(h) At each collection site, record the number of larvae in each dip, the number of dips taken, and the date and exact location.

(i) Larvae can be transported to the laboratory in glass or plastic specimen tubes (75 x 25 mm), in larger containers such as Mason jars, or in plastic self-sealing plastic bags. Plastic bags are much better, because the larvae sustain less damage as they hit the soft plastic walls during transport. An

ideal way to get mosquito larvae back to the laboratory alive, even over a rough road, is to place each sample in a plastic bag filled approximately 2/3 full and carefully sealed ("whirl packs" used for water samples are ideal), then float the bags in a cooler 1/4 filled with water. Collections should be protected against high temperatures and direct sunlight.

c. Mosquito Adult Surveillance.

(1) Background. Adult surveys are most frequently conducted because adult mosquitoes are generally easier to survey, collect, and identify than the immature stages. Light traps are limited to gathering data on density and species composition of nocturnal adult mosquito species that are attracted to light. Wide differences in capture efficiency have been noted between species due to differences in their reactions to light. Some species are caught in great numbers while others are rarely taken even though they may be plentiful in the vicinity (i.e., *Ae. aegypti* mosquitoes). Because of these behavioral differences, other types of adult mosquito collection methods (i.e., resting stations or landing counts) are needed to obtain a valid index of the total population.

(2) Purpose. Light traps are limited to gathering data on density and species composition of nocturnal adult mosquito species that are attracted to light. Some *Anopheles* and *Aedes* mosquitoes are poorly attracted to light, therefore light traps are ineffective in collecting these species. Although light traps are generally not recommended for use in collecting these genera, some *Aedes* and *Ochlerotatus* are strongly attracted to light traps (i.e., *Ae. vexans, Oc. sollicitans* and *Oc. taeniorhynchus*). *Anopheles* mosquitoes can be collected in inside huts in light traps that are on a level with the eaves. However, this method does not adequately estimate the risk of human-vector contact.

(3) Procedures for adult mosquito collections using light traps.

(a). Use three light traps at each location suspended 1.5-2 meters (4-6 feet) above the ground. Consider two traps as the minimum number if resources are constrained.

(b) Operate light traps two to four nights per week (minimum is two nights per week). Collection results from four nights will usually give as valid an index as seven nights per week. Light traps should be operated during the hours of darkness (from one hour prior to dusk until one hour after dawn).

(c) The best light trap collections are made during the new moon phase (no moon) or on overcast nights. Catch size is reduced markedly by competing light sources (street lights, perimeter lights, etc.). Mosquitoes come to lights in greatest numbers when nights are cloudy and humid and in smaller numbers when nights are clear and the moon is bright.

(d) Light trap placement is extremely important. Light traps should be placed between larval habitats and the area(s) to be protected, such as troop billets, housing areas, and bivouac areas. The trap should be placed along the edges of habitats to increase trapping efficiency and where the light is visible from all directions. The following areas are desirable for light traps locations: areas with low shrubbery and some shade in the vicinity; woods and swamp margins, secluded or semi-secluded areas away from traffic; accessible areas. Avoid areas near competing sources of artificial light, areas exposed to strong winds, near buildings housing animals, open water or open pastures, obstacles that block the trap's light, and areas where the trap is exposed to vandalism. Sometimes, moving a light trap a few meters can significantly change the number of mosquitoes collected. If a trap consistently fails to catch mosquitoes, it should be moved to another location.

(e) Portable mosquito light traps run various types of batteries. Light traps will run for more than one 12-hour night without recharging, IF the batteries are new. However it is best to exchange or recharge the batteries after every night's use.

(f) Light trapping should not be conducted on windy evenings. When wind speeds exceed 10-15 mph. mosquitoes do not generally fly.

(g) Rainfall during the night generally does not reduce the light trap catch. However, temperature plays an important role in the effectiveness of light traps. Many night flying species are completely inactive when the temperature between sundown and midnight is below 50^0F. Greatest activity takes place when the evening temperatures are 70^0F or higher.

(h) Unbaited light trap collections correlate poorly with human biting risk. Traps baited **with dry ice (CO_2) can predict 60-70% of the actual human biting risk** by some mosquito species (e.g., *Aedes* spp., *An. quadrimaculatus*, *Cq. perturbans,* and *Cx. salinarius).* This method collects significantly more with a greater variety of female mosquitoes compared to unbaited light traps. Dry ice-baited traps rely on CO_2 chemotaxis by host-seeking females and therefore are less influenced by background illumination. If available, bait light traps with a 1-2 pound (0.5 to 1 kg) of dry ice block (about the size of a brick) placed inside a padded envelope (or wrapped in several layers of newspaper) and suspend just below the cover of the light trap. A 5-pound (2 kg) block of dry ice may be needed to cover the normal dusk to dawn trapping period, if no insulation is used. **Dry ice can increase the overall catch of mosquitoes by up to 500% and can increase the number of species collected by 20-25%.** NOTE: remove the light source when dry ice is used as an attractant; the absence of light will eliminate other unwanted ("trash") phototactic insects from the collection and increase the efficiency of identification. Currently, a chemical source of carbon dioxide is under development by an industrial partner of the Army.

(i) Black-light (ultraviolet) lamps often attract a greater variety and increased numbers of mosquitoes.

(j) The chemical attractant octenol is not recommended. Octenol not significantly increase light trap collections for most mosquito species.

(k) Notify military police concerning appearance and exact locations of the installed light traps.

(l) Collect the traps as early as practical the next morning. Remove the mosquito-collecting bag containing the captured mosquitoes from the trap and loosely knot the sleeve. Then gently collapse the bag and place inside a box or chest. It is important not to crowd the collecting bags by placing too many in the chest, as crushing will kill the mosquitoes. The best way to preserve the characters on the adult mosquitoes is to hang the bags during transport, never allowing the bags to collapse. To kill the mosquitoes, they can be frozen with the rings of the bag propped apart to prevent contact between the netting and the adults or the bag can be hung inside a large plastic bag with vapors of ethyl acetate.

(m) Take the bags of mosquitoes to a convenient work site (field lab or merely a shaded place and vehicle tailgate) for rough sorting and storage. Anesthetize the mosquitoes with dry ice, ethyl acetate or by freezing. Untie the sleeve and gently shake out the mosquitoes on a light-trap lid for sorting. The mosquitoes can then be quickly sorted from the “trash” insects by aspirator or forceps and placed in flat-bottomed vials and tightly plugged with a rubber stopper or screw-top cap. Be sure to attach an adhesive label to each vial giving date and location.

(4) Protected human-bait human bait landing counts.

(a) Landing counts on humans are useful for determining population densities of anthropophagic (human biting) mosquitoes not attracted to light traps and for rapid checks of mosquito populations. The use of this method is recommended when complaints or suspicions do not reflect light trap collections. This survey technique establishes an index or landing rate by counting the number of mosquitoes landing on the collector during a specific period of time. However, landing count surveillance is time intensive, inconvenient, difficult to standardize, and poses potential health risks to the collector.

(b) **CAUTION!** This technique may increase the exposure of survey personnel to disease. Therefore, during a mosquito-borne disease outbreak use personal protective measures such as wearing headsets and rolling down sleeves, but do not use repellents. All survey personnel should be on any chemoprophylaxis recommended in the area being sampled. If a mosquito-borne disease is present for which no vaccine, chemoprophylaxis or treatment is

available (e.g., many viral diseases), this sampling technique should not be used.

(c) Conduct landing counts at three stations once every week (minimum requirement). These can be performed in houses, on porches, in village compounds, on farms and in forests, during the entire, or parts of the day and night; the place and time depending on the ecology of the mosquitoes. Make the landing counts at the same time of day (dawn, dusk, or other time depending on peak biting activity) to ensure uniformity. Use a flashlight when collecting mosquitoes.

(d) The technique used depends on the mosquito's biting habits. For some mosquitoes present in large numbers (i.e., salt marsh *Aedes*), 1-minute collections per hour are sufficient; however, since some mosquitoes may be "cautious" if present in low numbers, 10 to 15 minute collections per hour may be required. Take at least three counts at each station to obtain greater accuracy in estimating population size.

(e) One person can perform landing counts. Two persons are preferred for safety and effectiveness. Sit on a box or stool at the selected location with trouser legs rolled to the knees and socks rolled to the ankles. Collect mosquitoes that land on the exposed skin during the established time period. For consistency of results, the same person or persons should perform landing counts. Collections should be made with a battery-powered aspirator or a mouth aspirator fitted with a HEPA filter.

(5) Daytime resting station (natural and artificial) collections.

Many species of mosquitoes are weakly attracted to light traps, but can be easily collected from various daytime resting sites. Many species of *Anopheles* (malaria vectors) and *Culex*, and to a lesser degree members of other genera, rest during the daytime in relatively dark, humid shelters such as buildings, culverts, hollow trees, caves and underneath rock ledges and overhanging banks along streams. A careful inspection of their natural resting stations usually reveals resting mosquitoes and helps in estimating population density. Specimens can be captured in these shelters with the aid of a mouth-aspirator or motor-powered aspirator. The use of artificial resting stations (e.g., small wooden boxes, privy-type houses) is not recommended during deployments. Construction of the artificial resting boxes can be time consuming and they can be difficult to maintain. Alternatively houses or bedrooms can be space-sprayed with 0.05-0.1% pyrethrum, and the knocked-down adults collected from white sheets spread over the floor and furniture.

(a) A minimum of five resting stations (either natural or manmade) per installation or camp is recommended. Collections should be conducted a minimum of two days per week.

(b) Make these collections at the same time of day (preferably during the morning hours).

(c) Examine areas near the ceiling and floors and collected mosquitoes using an aspirator and a flashlight.

(6) Sweep-netting vegetation or use of motor-powered aspirators.

Both females and males can be collected by sweep-netting vegetation or using motor-powered aspirators. An aerial net may be used in this way, but the more durable sweeping net is recommended for such rough usage. The catch may be transferred from the bag to a killing jar in one of two ways. Single specimens are transferred most easily by lightly holding them in a fold of the net with one hand while inserting the open killing jar into the net with the other. While the jar is still in the net, cover the opening with the lid until the insect is "knocked down". When numerous specimens are in the net after prolonged sweeping, it may be desirable to put the entire tip of the bag into a large killing jar for a few minutes to stun the mosquitoes. They may then be removed and desired specimens placed separately into a killing jar, or the entire mass may be dumped into a killing jar for later sorting. This method of mass collecting is especially adapted to obtaining small insects not readily recognizable until the catch is sorted under a microscope and for the novice collector. Mosquitoes can be aspirated from under tree buttresses, bridges and culverts, and from rodent burrows, crab-holes, tree-holes, granaries and other shaded areas.

5. Surveillance data and action threshold.

a. The adult mosquito index is usually calculated to average several collection sites or traps to provide a composite index for a particular area or installation. One index commonly used for light trap collections and similar collection methods is:

$$\textbf{\textit{Adult Female Mosquito Index}} = \frac{\text{Total \# of female mosquitoes}}{\text{Total \# of traps x Total \# of nights}}$$

An alternative and more comprehensive index frequently used is:

$$\textbf{\textit{Adult Female Mosquito Density Index}} = \frac{A + B + C}{10}$$

A = light trap average (light traps operated at least twice a week)
B = landing rate average x 10 (landing stations visited daily with an average based on 2 or 3 day intervals)
C = daily complaint average (daily complaints based on 2 or 3 day intervals)

These data are generally plotted on a graph to help visualize changes in the mosquito population and detect long-term trends. These two indices can be

used to establish treatment thresholds (that level at which treatment is warranted). **In general, an adult female mosquito index of 10 or more usually indicates that treatment is necessary.** NOTE: treatment thresholds are only guidelines and will vary depending on the severity of the mosquito population, threat of vector-borne disease, size of the area, population involved and tactical situation.

b. Assessing larval population at sampling sites.

SEQUENTIAL SAMPLING TABLE

	Cumulative Number of Mosquito Larvae		
Number of Dips	**Low**	**Moderate**	**High**
1			> 31
2			> 36
3			> 41
4		2 - 3	> 46
5		4 - 5	> 51
6		5 - 7	> 56
7		7 - 10	> 61
8		7 - 15	> 66
9	1	7 - 20	> 71
10	1 - 2	7 - 30	> 76

Thus, the surveyor using this method ranks the pool sampled: i.e., If the number of larvae collected in at least 5 dips is 31 or more, the site is rated as "high".
If only 1 or 2 larvae are collected in 10 dips, the site is rated as "low".
If no larvae are collected, the site is rated as "nil".
10 dips must be taken to distinguish between "moderate' and "high".
Note: If the surface area of the larval breeding site is greater than 50 m by 50 m (2500 m²), the number of dips taken must be doubled.

c. Larval Indices.

Surveillance programs for dengue often estimate mosquito density based on larval frequency. The **Breteau Index** is a common expression of this type and is the total number of water containers positive for *Ae. aegypti* larvae per 100 houses sampled. There is a risk of dengue transmission when the Breteau index goes above 5, and emergency vector control is indicated when the index exceeds 100. Also, the **House (=Premise) Index** has been used for many years and is probably the most valuable and widely used single index. This is the percentage of houses (or tents or barracks in a military situation), including examination of surrounding compound, that have larvae of *Ae. aegypti* in at least some containers. The block index, which is the percentage of blocks of

houses that have houses where breeding is occurring, has also been used, especially in control campaigns where areas are divided into blocks for the convenience of spraying. Another index used is the **Container (=Receptacle) Index**, which is the percentage of water-holding containers examined that contain *Ae. aegypti* larvae. It has been shown that the population levels of *Ae. aegypti* as shown by the container, house, infested receptacle, and block indices are usually similar. In fact, there is a good positive correlation between all four indices, the best being between the house and block indices. Using a logarithmic transformation two regression formulae were devised: (1) House Index = antilog (0.0861 + 0.01586 x Block Index) and (2) Block Index = 1.50 + log 52.40 x log House Index. Thus if one index is obtained a very good approximation of the other can be calculated.

Recently, convincing evidence has been developed that the most accurate method of surveying *Ae. aegypti* is to count all pupae in every container of a series of houses. The number of pupae per person is calculated and can be translated to the number of adult female mosquitoes present. Unfortunately, this method would be very difficult to perform under military contingency conditions. In a military cantonment, it is assumed that larval sources of *Ae. aegypti* will be eliminated or treated, therefore the major source of vectors will be females flying from outside the cantonment. Under these conditions, surveillance for adult vectors is of primary importance and can be achieved through landing collections, by the use of a power aspirator, or indirectly by detecting eggs in ovitraps.

d. When to begin a control program. The question as to **when to begin a mosquito control program** can be addressed in 2 ways, depending on whether the mosquito problem is based on nuisance- or health-related concerns. If the problem is with nuisance mosquitoes, certain criteria are used and the emphasis is placed on prevention and mosquito larviciding. If there is a perceived risk of mosquito-borne disease, then other criteria are used to determine if and when a large-scale mosquito adulticiding program is necessary.

e. Management Aimed at Control of Nuisance Mosquitoes. The best time to begin a mosquito management program is immediately after the mosquito eggs have hatched, the pool has been inspected, and the numbers of larvae present justify the use of an insecticide, based on sequential sampling and pool ranking (i.e., low, medium, or high). When the larvae are concentrated in their breeding site, they are most efficiently and economically destroyed. Preventing the larvae from completing their development minimizes the area that would have to be treated and prevents an annoyance problem from developing. Larval breeding site maps are never complete. Intensive surveys (on foot, by vehicle and by air) during the first few months of a program should determine where most (85% or more) of the breeding sites occur. It takes repeated surveys to gradually find the next 5-10% of the sites. The last 5% will probably never be known. They may be small and inaccessible or situated in environmentally sensitive locations that cannot be treated. If 95% of the sites

are treated, larvae killed before they pupate and the adults emerge, the larviciding program should be considered to be excellent.

However, if mosquito larviciding has failed to control the larvae present or if adult mosquitoes have blown in from outside the larviciding zone or if funds did not permit an adequate larviciding program and the adult mosquitoes reach annoying levels, as determined by mosquito light trap collections and/or mosquito biting counts, then mosquito adulticiding is normally started. Mosquito light traps are an objective measure of mosquito activity. The mosquito light trap collections should average > 25 adult female mosquitoes per trap per night for 3 consecutive nights. This value recognizes that some traps may collect low numbers and others high numbers of mosquitoes on any given night. It also recognizes that mosquito activity may be unusually high or low, depending on weather conditions, on any given night. Mosquito biting counts can also be used as an index of mosquito activity. The counts, carried out according to standard methods, should average > 1 bite per minute over a 10 minute period at sunset before mosquito adulticiding is started. These values, for trap and landing/biting counts, are generally comparable. They also correlate well with the public's tolerance of nuisance mosquitoes. After these values are reached, the public usually begins to demand an adult mosquito control program.

f. Management Aimed at Control of Disease-carrying Mosquitoes. If there is a risk of mosquito-borne disease, the criteria used are different, reflecting the fact that the mosquito control effort will encompass a much larger area than routine mosquito control operations, that more people will be affected, and that the spray operations will cost considerably more. The decision to implement a large-scale, emergency mosquito management program will be based on an assessment of several variables. Mosquito surveillance will center on the known vectors of disease. In addition, surveillance will involve extensive monitoring of humans (e.g., Disease Non-Battle Injury rates) and animals (e.g., horses and sentinel chickens for virus activity). The decision-makers will also consider weather forecasts and the risk of disease transmission through to the end of the mosquito season.

Mosquito traps will monitor the fresh emergence of nearby male and female mosquitoes. If males appear in high numbers within the control zone, soon after larviciding, some major sites were missed. If high numbers of female mosquitoes are caught in the traps or landing-biting collections a week or so after the emergence should have occurred, this does not necessarily indicate a poor larviciding program. High winds may have driven flying mosquitoes into the treated zone. Or, perhaps, the buffer zone around the area is not large enough and the control zone must be expanded to minimize the inward migration of mosquitoes. As a rule of thumb, in open areas, the larviciding zone should extend at least 8 km beyond the built-up area of the base camp, city or town. Ideally, it should extend 24 km beyond the boundary of the occupied area.

6. Preservation.

a. Eggs

(1) Check with the laboratory that will receive the eggs to obtain necessary shipping permits or special handling procedures required when shipping live specimens.

(2) Dry the strips and mail them within 24 hours of the time they are collected. Time is crucial since the laboratory must hatch the eggs and rear the larvae to fourth instar to make a positive identification.

(3) Pack the velour strips as follows:

(a) Cover a piece of bond paper with a dry paper towel.

(b) Staple strips to the paper towel with the rough or egg side up (be sure the strips do not touch).

(c) Cover the strips with another sheet of bond paper.

(d) Place the paper “sandwich” inside a padded mailing envelope.

(4) Enter all necessary data on the label: date of collection, location, number, collector, etc.

b. Larvae.

(1) Process, record, and ship larval collections within 24 hours of collection. Accumulated collections cause unnecessary lag between the day of collection and receipt of identifications. Ship fourth instar larvae whenever possible. This makes identification easier and more accurate.

(2) To process larval and pupal collections before shipping:

(a) Ship fourth instar larvae whenever possible. This makes identification easier and more accurate. Whole larvae and pupae are preserved by first transferring them to a beaker of hot water. The hot water fixes the proteins that prevents later darkening of the specimens. DO NOT use boiling water; hot tap water is sufficient. Hot water causes the larvae to expand so external features are easy to observe for identification. If no hot water is available, larvae may be transferred directly into alcohol.

(b) After they are killed and floating on the surface, transfer the larvae in vials containing 80% ethanol. The ethanol should be replaced at least twice to eliminate excess water. Larval mosquitoes may also be preserved in a

mixture of 5 parts 70% isopropyl (=rubbing) alcohol and 1 part glacial acetic acid. This works well to preserve larval hairs and prevent dehydration of the specimens. For short periods of preservations, 70% isopropyl alcohol can be used. During WWII, some entomologists even used after-shave lotion or whiskey to preserve mosquito larvae in an emergency.

(3) The transfer may be made in the field directly from the dipper by carefully using an eyedropper to transfer the larvae, with as little water as possible, into prepared tubes. If specimens are too large to pass through the narrow opening of an eyedropper, do not force them, simply reverse the bulb on the tube using the larger opening. Another method is to use the flattened end of a stick to gently lift the specimen from the hot water and place it directly in the alcohol solution. This method also avoids the need to change alcohol solutions to eliminate water introduced with the specimen.

(4) Completely fill the tubes or vials with the alcohol to eliminate all bubbles thus preventing specimen movement and damage. This can be done by introducing a plug of alcohol-soaked cotton above the specimen in the tube. Another method is to use a narrow vial with a rubber top that can be pierced with a hypodermic needle. The tube is filled completely with alcohol and the top put in place while piercing it with the hypodermic. Pressure in the vial is relieved through the needle and no air gets into the vial. Larval mosquitoes may need to be mounted on slides and examined under a compound microscope for confirming the species identification.

(5) Individually wrap the glass tubes with cotton before placing them in the mailing case. Use mailing case liners (e.g., bubble wrap) to reduce shock to the specimens.

(6) Prepare a pencil label and place it in the vial for each collection site. Make the label large enough not to move around inside the vial, but small enough not to be stuffed. Be sure that the label will not bounce against the specimen during shipment. The label should either be above the cotton plug or made large enough that it is wedged inside the tube.

(7) Enter all necessary data on the label: date of collection, location, number, collector, etc.

c. Adults.

(1) Adults are killed using ethyl acetate. Glass killing tubes with the bottom 1/3 filled with oven-dried plaster of Paris are used. The plaster is saturated with the ethyl acetate, but not to the point of leaving excess liquid. As the mosquito dies its legs will usually relax away from the body. In this position, it is much easier to affix the specimen to a paper pin, if desired. To pin adults, attach paper points to No. 3 stainless steel insect pins.

(2) Sort, record, and ship the light trap specimens on the day that they are collected. Process, record, and ship landing collections within 24 hours of collection. Accumulated collections cause unnecessary lag between the day of collection and receipt of identifications. Enclose a card with the date of collection, location, trap number, collector, etc. inside the container. Individually wrap each container before placing them in the mailing case. Adult mosquitoes should not be preserved in ethanol or other liquids.

(3) To separate mosquitoes from other entomological specimens and debris:

(a) Empty the contents of the collection bag on a sheet of white paper.

(b) Use fine forceps to sort mosquitoes. Great care is needed not to denude the mosquitoes of scales as in many species these are critical for identification. Mosquito species vary greatly in size; therefore, do not rely on size alone for sorting out and making a preliminary identification of adult mosquitoes. There are many insects that closely resemble mosquitoes, such as smaller flies and midges. When in doubt on whether a specimen is a mosquito or not, include it with the group of insect specimens to be sent for identification.

(4) The following detailed instructions for packaging adult mosquitoes for shipment is from the publication "**Guidance For Packaging Of Light Trap Mosquito Specimens For Identification**", U.S. Army Center For Health Promotion And Preventive Medicine-North (March 8, 2000).

(a) Try to separate and remove non-mosquito specimens (e.g., moths, beetles, and flies). Spreading them out in a flat white enamel pan and sorting them with a fine camel hair paintbrush can make the job easier (Note: if you tilt the pan and tap it lightly, the beetles usually roll to the low end). If you are not sure if it is a mosquito, include it in your shipment. You should count the female mosquitoes, prior to packing, and provide this information to the installation pest controller for use in control decisions.

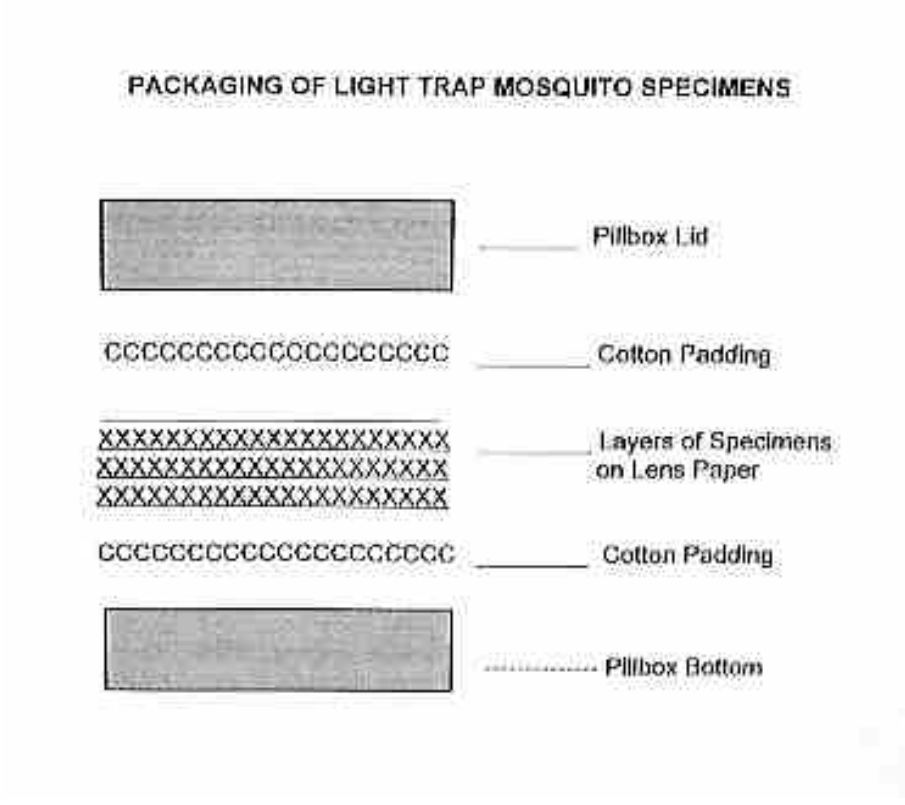
(b) Place light trap specimens between layers of soft, light, smooth paper. Lens paper is ideal. Do not use cardboard, writing, or typing paper, as they are heavy and will crush the specimens. Do not put specimens directly on cotton, as they get tangled in the fibers and cannot be removed intact for identification.

(c) The layers of soft paper should be cut slightly larger than the pillbox. This can be easily done by placing the top of the pillbox on an empty inked stamp pad, pressing the top of the pillbox on the paper and then cutting with scissors around the impressions.

(d) Try to arrange specimens so they do not overlap and are not entangled. First, put a small piece of cotton in the bottom of the pillbox to act as a cushion. Then add a layer of soft paper, a layer of specimens, a layer of paper, a layer of specimens, a layer of paper, a layer of specimens, a layer of paper, and finally a small piece of cushioning cotton. This results in the packaging of a total of **three** layers of specimens. Please refer to the diagram on the following page.

(e) A separate pillbox should be used for specimens collected in one trap, which, in turn, had run for one night. Be sure each pillbox has the date of the night trapping took place and the trap identification code or name. If more than one box is used for a large single trap catch, then those boxes should be numbered consecutively on the outside of each pillbox (e.g., "1 of 3", "2 of 3", "3 of 3").

(f) Be sure to completely fill out printed data labels on the pillbox tins. If you do that, no other paperwork is needed. If you do not have the tins with labels, please fill out the "Adult Mosquito Identification" card [CHPPM Form 189/CHPPM Form 8023 (Modified), Feb 97] for **each** trap collection. Instructions are printed on the back of the card. If this form is not in hand, use DA-Form 8023-R (Adult Mosquito Collection), adding the trap location in the Remarks section, or 8022-R (Adult Mosquito Identification) leaving the species identified sections blank. These forms can be copied from the masters provided at the back of TB MED 561.



(5) If the package is to be mailed internationally, it should have the necessary customs declaration form and the contents should be identified as "DEAD INSECTS FOR SCIENTIFIC STUDY – OF NO COMMERCIAL VALUE."

CHAPTER 5: Biting Midges (Family Ceratopogonidae)

1. Military Importance.

These blood-sucking midges can transmit filarial parasites and viruses to humans. However, their greatest impact on military operations comes from their potential for huge populations and the characteristic burning and itching associated with their bites, often causing intolerable annoyance. Work and sleep can be almost impossible in areas with large adult female populations, due to their voracious blood feeding. Local reactions can be severe enough to render a military unit operationally ineffective.

2. Background.

Biting midges in the genera *Culicoides* and *Leptoconops* contain species that are pests of man and livestock. Culicoides are known vectors of filarial parasites (*Acanthocheilonema perstans* in Africa and *Mansonella perstans*), protozoan (blood parasites of birds) and viral (Oropuche virus and suspected in eastern equine encephalitis transmission in man and bluetongue transmission in cattle) diseases of medical and veterinary importance throughout the world. Some species of *Culicoides* and *Leptoconops* are responsible for disease conditions caused by sensitization to their bites. Several species of biting midges can also be a considerable biting nuisance. The tremendous numbers of *Culicoides* midges that are present in coastal areas can make life almost unbearable in some places by their annoying attacks.

Biting midge larvae occupy a wide variety of aquatic, semi-aquatic and terrestrial habitats. However, there is great diversity of larval breeding habitat. Some occur in soil, from beach sand with low humus content to highly organic muck. Others are found in coastal saltwater beaches, tidal pools, and ditches; inland fresh water bogs, marshes, swamps, rice fields, pools, tree-holes, and small streams. Immatures most often are collected from the surface of gently sloped mud that is open to sunlight near the edges of impounded or slowly moving water. However, this method involves collecting mud samples and extracting the larvae by a flotation method in the laboratory. Therefore, this method is impractical for use in the field.

3. Geographic distribution and habitats.

Biting midges are considering the most troublesome outdoor pests of both temperate and tropical regions, due to their high populations at times and their painful, “burning” bites. Biting midge larvae can develop in nearly any kind of moist, organically enriched, muddy substrate. Larvae are neither strictly aquatic nor terrestrial, but they cannot develop without moisture. Many species breed on the shores of streams and ponds in muddy or sandy substrates. Salt marsh and mangrove habitats are special breeding sites for the most pestiferous

species of coastal midges. Breeding may occur year around in the tropics. In temperate regions, biting midges mostly overwinter as larvae (although a few species may overwinter as eggs).

4. Procedures.

a. Flotation of larvae and pupae with salts. This method is very time consuming and larvae are difficult to identify. This survey method is generally not recommended for deployments.

(1) Place mud sample in container with fresh water and agitate. Allow agitated samples to settle overnight.

(2) Sieve the water and top portion of mud and place the residue retained in a 100-mesh screen into a saturated solution of magnesium sulfate ($MgSO_4$) to "float" the larvae to the surface where they are removed with a pipette. Note: Ordinary sugar or salt in nearly saturated solutions can also be used to float larvae and pupae.

(3) This method or direct flotation of the entire sample can be used to collect larvae.

b. Collection of larvae and pupae using a Berlese Funnel. A Berlese funnel can be used to dry samples of suspected breeding media. The larvae and pupae migrate downward as the surface dries and are collected in a jar of 70% ethanol beneath the funnel. Although there is some mortality due to larvae drying out while crawling down the sides of the funnel, large numbers of larvae and pupae can be collected.

c. Collection of Adults with Light traps. In general, biting midges are best sampled with light traps. Light traps can provide a rough estimate of the species in the area. Many species are up to 10 times more attracted to black light than to incandescent light. Carbon dioxide (usually dry ice) will also increase trap collections for some midges. The carbon dioxide, perhaps perceived as host exhalations, serves as an attractant for females seeking a blood meal. However, this attractant is not effective in attracting gravid females. The particular advantage of carbon dioxide-baited traps is the collection of daytime (diurnal) species. Light traps near animal pens produce numerous recently blood-fed insects, providing useful data on feeding habits if combined with blood-meal analysis. In general, the more powerful the light source, the greater will be the number of midges caught; black-light traps will generally attract greater numbers than tungsten lamps. The following procedures should be followed for a surveillance program.

(1) Trapping should be conducted when the average wind velocity is 5 mph or less;

(2) The temperature is between 75^0 and 95^0 F;

(3) The relative humidity is 60% or less;

(4) Traps should be placed 50 or more feet apart with trap centers 4 feet above ground level;

(5) The use of dry ice as an attractant with light traps, sticky traps and suction traps will increase catches of certain species of both *Culicoides* and *Leptoconops*. If dry ice is used, each trap should be stocked with 2.5 pounds (1 kg) of dry ice.

(6) Because of their minute size, light traps used to collect biting midges must be equipped with collecting nets constructed with very fine-mesh fabric (20 squares per running cm; 52 squares per running in.).

d. Hand-operated sweep nets. The sampling technique is useful in obtaining adult flies for identification, but quantitative estimates of the fly population are difficult to obtain and unreliable. A fine mesh net must be used because of the small size of the adults. Efficient use of the net is gained only with experience. Collection of specimens in flight calls for the basic stroke – swing the net rapidly to capture the specimen, then follow through to force the insect into the very bottom of the bag. Twist the wrist as you follow through so the bottom of the bag hangs over the rim; this will entrap the specimen.

e. Sticky traps (adhesive papers). Papers coated with castor oil fastened on boards at various heights above ground level can be superior to light or landing collections for measuring densities of many adult *Culicoides* spp. These adhesive papers can also be used to make “sticky cylinder traps” consisting of sheets of adhesive-coated paper or plastic film clipped to cylinders fastened at various heights to posts. A useful and inexpensive variation of this method is the “paddle trap”, consisting of a square of mosquito screening greased with castor oil and mounted on a wooden stick.

f. Mouth/mechanical aspirator (protected landing collections). See procedures for adult mosquitoes.

g. Malaise traps. The use of Malaise traps is not recommended to collect adult biting midges.

5. Surveillance data and action threshold.

An adult biting midge index (similar to adult female mosquito index) is usually calculated to average several collection sites or traps to provide a composite index for a particular area or installation. One index commonly used for light trap collections and similar collection methods is:

$$\textit{Adult Biting Midge Index} = \frac{\text{Total \# of biting midges}}{\text{Total \# of traps} \times \text{Total \# of nights}}$$

An alternative and more comprehensive index frequently used is:

$$\textit{Adult Biting Midge Density Index} = \frac{A + B + C}{10}$$

A = light trap average (light traps operated at least twice a week)
B = landing rate average x 10 (landing stations visited daily with an average based on 2 or 3 day intervals)
C = daily complaint average (daily complaints based on 2 or 3 day intervals)

These data are generally plotted on a graph to help visualize changes in the biting midge population and detect long-term trends. These two indices can be used to establish treatment thresholds (that level at which treatment is warranted). **In general, an adult biting midge index of 10 or more usually indicates that treatment is necessary.** NOTE: treatment thresholds are only guidelines and will vary depending on the severity of the mosquito population, threat of vector-borne disease, size of the area, population involved and tactical situation.

6. Preservation.

All stages are normally preserved in 70-80% ethanol for identification purposes. For larval preservation, see procedures for larval mosquitoes for details. It is usually necessary to mount and dissect adult biting midges on microscope slides for accurate identification under the higher magnification of a compound microscope.

Before adults are preserved in ethanol, they should be collected from the collection bag with a mouth aspirator and blown directly into a jar containing water or normal saline (0.9%) with a little (0.01%) detergent to wet insects. Do not leave in detergent solution as insects in detergent decompose very rapidly in tropical daytime temperatures and become useless for detailed study. Do not fill the container to the top with specimens, as they will rot from insufficient alcohol. Vials should never be filled more than one-third full with specimens. Midges for taxonomic study are best slide-mounted after dissection, preferable with wings, head, abdomen and thorax (legs attached) under separate small cover slips.

Adult midges can also be collected dry and packaged in tissue paper for shipment. For virus isolation studies, they should be collected dry, killed by freezing in a -70^0C freezer and sorted rapidly over ice. Dry ice is best avoided, since the low pH induced by carbon dioxide can kill any virus in the insects. Liquid nitrogen is ideal for preserving insects for virus studies but supply, storage and transport of this substance may present significant problems under deployed conditions.

CHAPTER 6: Black Flies (Family Simuliidae)

1. Military importance.

Black flies are vicious and persistent biters causing swelling and pruritis. The bites will typically cause considerable pain and irritation, and may frequently give rise to secondary infection. Species of *Simulium* are intermediate hosts for the filarial worm, *Onchocerca volvulus*, the organism causing onchocerciasis in certain tropical areas. This disease may not have any short-term effect on military operations, but long-term health effects can be serious.

2. Background.

Black flies are important vectors of onchocerciasis ("river blindness") in tropical Africa roughly between 15^0 N and 12^0 S latitudes; tropical America from southern Mexico to Columbia, Venezuela, and northern Brazil. Onchocerciasis is known to be endemic in 37 countries. In 1995, the WHO estimated that 123 million persons were at risk of contracting the disease, and 17-18 million were infected, of whom about 270,000 were blind and another 500,000 severely visually impaired. About 95% of infected people reside in Africa, where the disease is most severe along the major rivers in 30 countries in a belt spanning the northern and central part of the continent. Outside Africa, onchocerciasis occurs in Mexico, Guatemala, Ecuador, Colombia, Venezuela, and Brazil, and Yemen, Asia. Besides being disease carriers, black flies are annoying to humans and domestic animals because of their biting and crawling habits.

3. Geographic distribution and habitat.

Black flies are found worldwide. However, the greatest number of species is found in a Palearctic region. Black fly larvae and pupae are found almost exclusively in relatively unpolluted temporary or permanent flowing waters. Some important disease vector species occur primarily in rivers. Pupae are found attached to the substrate in the same locations as the larvae. Females of many species are known to suck blood from mammals and birds.

Most black fly species are attracted to visual and chemical stimuli. Many black flies species are diurnal (active during the day) therefore, light traps are probably not an effective sampling method for these species. Many diurnal species of black flies are attracted to large, dark silhouettes (i.e., large animals and humans). Because of this behavior, daytime human landing count collections can be an effective means of sampling certain black fly species.

Carbon dioxide (CO_2) has long been used as an effective attractant to survey for many species of biting Diptera. While CO_2 is effective for black fly surveillance, it also has limitations from an operational point of view. Use of CO_2 as an

attractant entails use of either bottled gas for dry ice. While both sources of CO_2 are relatively inexpensive, their use and availability in remote locations can be problematic.

4. Procedures.

Collection of larvae and pupae is time consuming and may not be possible during deployments. Breeding habitats can be difficult to locate, and immature black flies are difficult to identify. However, if desired, larvae and pupae are best removed from their substrates with fine forceps, care being taken with pupae to apply a light side-to-side pressure with will loosen the cocoons and allow them to be picked off without being crushed. Or, larval collections are made in turbulent sections by picking the larvae from solid substrates (e.g., rocks and sticks) or by scraping the surface of submerged objects while holding a net downstream. Pupae are best collected by handpicking because they are usually firmly attached to the substrate. Examine 5-10 stones or sticks at each site, depending on the size of the stream and the availability of substrate.

a. Mouth/mechanical aspirating adults. Direct protected human bait collections conducted during daylight hours are likely to result in more representative samples of anthropophilic black flies than other methods. Human bait counts are the recommended surveillance technique for biting flies. Frequently, catches lasting 1-3 hours are adequate, but because biting times vary among different species it is important to ensure that these short-duration collections are made when the flies are actively biting. If catching extends over long periods of time (e.g., 0600-1800), specific collections can be made for only a short period of time (e.g., 15-30 minutes) each hour Catches can be performed from a single (or a few) stationary bait stations or made at several sites along a transect. “Nervous” or “shy” species, which are easily disturbed by slight movements and fly off again, are best collected by using a descending net. Dark skinned people are generally more attractive to black flies than light-skinned individuals.

b. Hand-operated sweep nets of adults. Adults all too frequently come to the collector and can be taken by sweeping a net around the head. Specimens can also be collected by sweeping vegetation in the vicinity of their breeding areas.

c. CO_2-baited light trapping of adults. Both males and females are attracted to CO_2-baited light traps. These baited light traps with the bulb removed are successful in catching a wide variety of black fly species. Trapping should be conducted during the hours of daylight (0600-1800 hours) when adult flies are active. Light trapping of adult black flies during the hours of darkness is generally not recommended as most species are diurnal. However, a few studies have reported that some species can be collected in light traps during the hours of darkness.

5. Surveillance data and action threshold.

Although the larval stage is the preferred stage for control, there is no generally accepted action threshold. This is due, in part, to the fact that threshold for a specific location, one should consider the number of people who will be affected and the environmental consequences of any action. However, it should be possible to develop an action threshold for larviciding using artificial substrates for monitoring the larval population. Larviciding might begin when 'x' number of larvae (of such pest species complexes) is collected from a given unit of substrate (e.g., 60-80 larvae per 60 cm x 1 cm polyethylene tape, exposed in the stream for 48 hours). In practice, such action thresholds will vary from region to region, depending on the species and diseases present, substrate used, and tolerances of the people affected. At one time, the Canadian Forces established an action threshold of 3-5 black fly bites per minute as the level at which to implement adulticiding of the entire installation. Currently, one must determine, from complaints and adult surveys, that a problem exists and rely on the larval surveys to locate the breeding sites and to decide on the best time for black fly larviciding, adulticiding, or both. This approach does not always work well. In many areas some species of black flies may fly into an area from a considerable distance. One must recognize that a black fly problem in one area may result from sources outside that jurisdiction. In such situations, management measures may be limited to adulticiding and personal protection and, consequently, provide only temporary relief. When comparing biting rates, it is usually important to sample adults at the same place and time, because most species exhibit periodicity of biting which may alter according to vegetation zone, types of habitat and season.

6. Preservation.

Larvae and pupae are normally preserved in 80% ethanol for identification purposes. Slide preparations of whole larvae and adults are useless, and specimens should never be sent to specialists as slide material. Adults can be preserved in alcohol, but this should not be done if identifications are needed; fluid preservation makes certain features difficult to see and gradually decolorizes specimens (thereby destroying important taxonomic features). Adults intended for identification should be micro-pinned through the center of the thorax. Flies should not be gummed onto a card point, stuck with shellac to the shaft of a large pin, or impaled on the tip of a micro-pin, as these methods make flies very vulnerable to loss or damage during shipment.

CHAPTER 7: Sand Flies (Family Psychodidae)

1. Military importance.

Phlebotomine sand flies are of medical importance in transmitting the pathogens that cause human leishmaniasis, Bartonellosis, sand fly fever and several viral infections. Although not as well known as dengue or malaria, these are severe diseases that are difficult to treat medically. Leishmaniasis and sand fly fever have been important in a number of military campaigns.

A total of 31 cases of leishmaniasis (12 cases of visceralizing *Leishmania tropica* and 15 cases of *L. major*) were diagnosed and treated from Gulf War veterans. Superficially this appears to be a small number of cases out of the total of approximately 550,000 veterans, and perhaps a minor issue. In fact, many millions of dollars were sent on this disease for treatment and research as part of the larger Gulf War Illness investigations.

2. Background.

Compared to other Diptera, sand flies are small (<5 mm long) and fragile. These tiny, densely haired flies characteristically rest with their wings held upward and outward above the body. This resting stance, coupled with a dark-gray to dusty-brown color, relatively large eyes, long antennae and fuzzy appearance give them an unmistakable appearance.

Sand fly bites are usually quite painful. Feeding activity is influenced by environmental factors such as temperature, humidity and air movement. Most human-biting species feed at dusk and during the evening, when temperatures drop and relative humidity rises.

3. Geographic distribution and habitat.

Sand flies occur mainly in the tropics and subtropics, with a few species ranging into temperate zones of the northern (to 50^0N) and southern (to 40^0S) hemispheres. Distribution is limited to areas that have temperatures above 15.6^0C for at least 3 months of the year. There are no sand flies in New Zealand or on Pacific Islands. Human-biting sand flies in the Old World are distributed mostly in the subtropics, with a few human-biters south of the Sahara and none in Southeast Asia. In the New World, they are limited mainly to the tropics.

Sand flies occur in a wide variety of habitats distributed in elevation from below sea level in areas surrounding the Dead Sea in Israel and Jordan to 2,800 meters above sea level in the Andes Mountains and Ethiopia. Immature stages (larvae and pupae) are found in organically enriched soil in a variety of habitats (e.g., animal burrows, tree holes and animal pens). In many arid or semi-arid

habitats (e.g., East African savannas), sand fly populations are highest toward the end of the rainy seasons and lowest toward the end of the dry season. In hot, dry deserts (e.g., Southwest Asia) or in dry temperate climes with hot summers and cold winters (e.g., southern Europe), adults of some species may disappear entirely during the driest and/or coldest seasons of the year.

4. Procedures.

a. Mouth aspirating adults. Protected human landing or resting collections can be used to collect sand flies from a variety of diurnal resting sites, including tree trunks, tree hollows, animal burrows and dens, rock crevices, termite mounds, and other protected resting sites. See procedures for adult mosquitoes for detailed instructions. Human landing collections will provide the best representation of anthropophilic species in a given area, but carry the greatest risk for acquiring a vector-borne infection. Adult protected landing rate counts is the recommended surveillance technique for sand flies, however if leishmaniasis is endemic in the area, very careful consideration should be given to the potential of technician collectors acquiring this potentially living threatening disease.

b. Hand-operated sweep nets of adults. Adults all too frequently come to the collector and can be taken by sweeping a net around the head. Specimens can also be collected by sweeping vegetation in the vicinity of their breeding areas.

c. Light trapping of adults. Both males and females are attracted to light traps. Light traps should be set up at a height of 1-1.5 meters from the ground, and will be more effective is used in conjunction with dry ice. A fine mesh collection bag should be used to ensure that no flies escape through the mesh screen.

d. Sticky traps (adhesive papers). Papers coated with castor oil fastened on boards at various heights above ground level can be superior to light or landing collections for measuring densities of many adult sand fly species. These adhesive papers can also be used to make “sticky cylinder traps” consisting of sheets of adhesive-coated paper or plastic film clipped to cylinders fastened at various heights to posts.

5. Surveillance data and action threshold.

Phlebotomine sand flies are rarely present in sufficient density to reach pest proportions, but their importance is as vectors of various pathogens, the most important of which are species of *Leishmania* causing cutaneous, visceral, and mucocutaneous leishmaniasis of humans. Therefore, even low densities of sand fly adults may trigger the start of control operations. Phlebotomines are highly susceptible to common insecticides and re-infestation is slow.

6. Preservation.

All stages of sand flies can be preserved in 80% ethanol for identification purposes. However, long-term storage of adults in alcohol discolors specimens and makes them difficult to clear for identification. Alternately, specimens can be stored dry in a vial under gentle pressure from a twist of tissue paper to avoid movement. Cotton should never be used as it can cause antennae, legs etc. to become entangled and liable to be broken off when specimens are removed. Sand flies usually need to be slide-mounted for accurate identification. For larval preservation, see procedures for larval mosquitoes for details.

CHAPTER 8: Tsetse Flies (Family Glossinidae)

1. Military Importance.

Tsetse flies transmit the trypanosomes that cause African sleeping sickness vectors throughout central Africa. The Gambian form of the disease (caused by *Trypanosoma brucei gambiense*) is transmitted by tsetse in the *palpalis* group (e.g., *Glossina palpalis palpalis*, *Glossina tachinoides* and *Glossina fascipes fuscipes*); the Rhodesian form (caused by *T. b. rhodesiense*) is transmitted mostly by tsetse in the *morsitans* group (*Glossina morsitans morsitans* and *Glossina morsitans centralis*). Military operations in central Africa would be greatly impacted by the threat of this disease due to the lack of vaccines and chemoprophylaxis.

2. Background.

Tsetse flies transmit the trypanosomes that cause African sleeping sickness vectors throughout Central Africa. The Gambian form of the disease (caused by *Trypanosoma brucei gambiense*) is transmitted by tsetse in the *palpalis* group (e.g., *Glossina palpalis palpalis*, *Glossina tachinoides* and *Glossina fascipes fuscipes*); the Rhodesian form (caused by *T. b. rhodesiense*) is transmitted mostly by tsetse in the *morsitans* group (*Glossina morsitans morsitans* and *Glossina morsitans centralis*).

Tsetse are flies of medium size, ranging from 6 to 15 mm in length. They are brownish in color; the body is somewhat wasp-like; and the wings, when at rest, are crossed scissor-like and extend well beyond the tip of the abdomen.

Both sexes feed avidly and exclusively on vertebrate blood and can transmit parasites equally. The flies are attracted to moving objects. They usually feed in broad daylight and bite humans easily through thin clothing. Sight apparently plays the most important role in host finding, although the olfactory sense may also be involved. Tsetse feed on a wide range of vertebrate hosts (mammals, reptiles and rarely, birds). Although humans are freely attacked by some tsetse, in general, humans are not the favored host. Tsetse blood feeding takes 1-3 minutes and the bite of the infective fly often remains unnoticed.

3. Geographic distribution and habitat.

Tsetse are almost completely restricted to continental Africa. However, some tsetse flies do occur on some small offshore islands such as Zanzibar, and there are 2 records of 2 species collected in southwestern Saudi Arabia. *Glossina* require shade, humidity and an outside temperature range between 20-30^{0}C to survive.

Species of the *Glossina palpalis* group are found primarily in wet lowland forests, especially along the shores of lakes and rivers, closely associated with riverine vegetation. These flies may become aggressive biters during periods of drought when water holes dry up and few such sites are available. Their distribution extends into adjacent savanna beyond forest boundaries along rivers and streams that flow into the Atlantic Ocean and the Mediterranean Sea, but not those that flow into the Indian Ocean.

The *Glossina morsitans* group is the most widespread, ranging through a large sub-Saharan belt across Africa from west to east. The cold winter temperatures of South Africa and the dry deserts of North Africa define the limits of this group. Flies of this complex are found in bushy thickets and are the usual savanna or game vectors of trypanosomes causing both Rhodesian sleeping sickness in humans and nagana in cattle.

4. Procedures.

Tsetse fly puparia can be collected by sieving sand or soil from their breeding sites. However, even under the best of circumstances it is a labor-intensive and tedious process, and not recommended during deployments.

a.. Mouth/mechanical aspirating adults. See procedures for protected landing collections for adult mosquitoes. Adult landing rate counts are the recommended surveillance technique for tsetse flies.

b. Hand-operated sweep nets of adults. Adults frequently come to the collector and can be taken by sweeping a net around the head. Specimens can also be collected by sweeping vegetation in the vicinity of their breeding areas.

5. Surveillance data and action threshold.

Tsetse fly transmission of African sleeping sickness is dictated by the presence of the vector, not so much by the abundance of flies but by the intensity of the human-fly contact which depends on the climatological and vegetational conditions, the availability of animals as food sources, the movement and habitats of humans. Therefore, preventive and control measures should be initiated even at very low adult tsetse densities.

6. Preservation.

Adult flies and puparia can be preserved in 70-80% ethanol but if identification by a specialist is required, at least some adults should be preserved as dry-pinned specimens.

CHAPTER 9: Other Biting Flies (Families Tabanidae & Muscidae)
HORSE FLIES AND DEER FLIES (Family Tabanidae)
STABLE FLIES and HORN FLIES (Family Muscidae)

1. Military Importance.

Tabanids (horse flies and deer flies) are of medical importance in two ways: 1) their bites may cause serious annoyance to humans and animals; and 2) they may serve as mechanical vectors and biological hosts of human and animal pathogens. They have the potential for transmitting loiasis in Africa, anthrax and tularemia to humans. Adult tabanids are persistent biters, and their painful bites are extremely annoying. Tabanids are difficult to control. Larval control is impractical, and ultra-low volume aerosols are generally ineffective against adults. Localized control can be achieved around military encampments using a variety of simple traps.

2. Background.

Only the female horse fly blood-feeds and seeks hosts during the day. Female tabanids have bladelike mouthparts that inflict painful, lacerating wounds and can cause considerable flow of blood. The puncture in the skin continues to ooze blood after the fly has fed. Tabanid bites often become secondarily infected, and systematic reactions may occur in hypersensitive individuals. Tabanids show two peaks of diurnal (daytime) activity. A minor peak extends from about 1 hour before sunrise to about 2 hours after sunrise. The major peak begins in late afternoon and builds to a peak at sunset after which activity drops off very rapidly. The period of least activity for all species falls between 1100 and 1500 hours.

The stable fly**,** *Stomoxys calcitrans*, and the horn fly, *Haematobia irritans*, are probably the two most common and widely distributed bloodsucking Diptera that attack domestic livestock. These two species are proven vectors of some diseases and potential vectors of others. They should definitely be controlled during an outbreak of foreign or endemic diseases.

The stable fly is similar in appearance to the house fly though they have a slender rigid proboscis projecting forward beneath the head. Both male and female stable flies will take blood from a large variety of animals including man. The stable fly can be an important human pest in beach areas where the fly breeds in fermented marine vegetation deposited above the normal high tide mark by high storm tides. Blood-feeding flies tend to concentrate on the lower portions of the legs, but feeding may occur on any part of the body. The fly only stays on the animal long enough (5-10 minutes) to obtain its daily blood meal. The fully engorged fly will then leave the host and finds a suitable resting place, commonly vertical surfaces such as a fence, sides of buildings, trees or bushes. Stable flies are strong fliers and may travel several miles in search of suitable

oviposition sites. Very good breeding places are afforded by leftover soggy hay or grain in the bottoms of or underneath out-of-doors feed racks in connection with cattle pens.

The horn fly, an obligate bloodsucking parasite of domestic livestock that feeds 20 to 30 times per day, is about one-half the size of the house fly. Both sexes take several blood meals per day. The fly remains on the host day and night, though the female may leave to oviposit. The fly deposits its eggs almost exclusively on freshly passed cow manure. Flies may travel up to 5 miles in search of a new host when disturbed. Ordinarily the flies congregate on the shoulders and sides of the animal, but may be seen on the underbelly during periods of heavy rain or extremely sunny and hot weather. Horn fly populations are most numerous during warm, moist weather and relatively scarce in hot, dry weather. The damage caused by horn flies is chiefly through irritation and annoyance. Man is only rarely attacked. A population of several thousand horn flies may be present on one animal. When large numbers of these flies are on cattle, the cattle bunch and expend considerable effort fighting the flies. They will often stand in water or seek shade trying to get relief from the flies. When they do this, they fail to graze normally.

Chemical attractants, such as acetone, octenol, and 3-phenyl-1-propanol, have been shown to be effective in collecting stable flies, tabanids, and tsetse flies. In fact, CO_2 and CO_2 with octenol-baited traps have been shown to collect over 300% more greenhead flies (salt marsh tabanids) than unbaited traps.

3. Geographic distribution and habitat.

The stable fly is cosmopolitan and has been reported from all geographic areas except the Polar Regions. The horn fly has a somewhat more limited range thought it is a pest of cattle in most of the world. Horn flies are well-established pests of cattle throughout most of Europe, North Africa, Asia Minor and the Americas.

Tabanids have an almost worldwide distribution, being absent only in high altitudes and latitudes and in such insular areas as Hawaii. Most species of horse flies and deer flies breed in aquatic or semi-aquatic habitats such as marshes and stream banks. Most temperate climate species produce one generation per year. In temperate climates, the females are only active during the warm summer months. In the tropics, activity may extend throughout the year.

4. Procedures.

NOTE: Survey collections of the immature stages of all these flies are not recommended because of the problems inherent in locating and removing the immature stages from the environment.

a. Adult Landing Counts (Visual Counts). Adult landing counts are an effective surveillance technique for biting flies during deployments. Estimates of adult horn fly populations are relatively easy to make because of the close association of the fly with the host. Counts can be made either with or without binoculars, though those made with binoculars are usually more accurate. Binoculars allow the survey person to "stand off" a short distance from the host and not disturb the flies. A total of 15 animals should be surveyed from a herd and an average number of flies per animals calculated. Stable flies are monitored by counting the flies on all four legs of about 15 animals.

b. Sticky traps. The most common method of estimating populations of stable flies on resting sites is to capture flies in sticky material such as tanglefoot. Traps used for this purpose are usually 25- by 30-centimeter plywood panels painted with adhesive and placed on vertical surfaces (about 130 centimeters above the ground) in areas where cattle congregate. Traps of sticky fly paper can also be used. Sticky traps cannot be used to quantify population levels; they are only useful in establishing the presence of the flies. Sticky traps can also be used to collect tabanids, however this survey method may be inefficient. Black is the most attractive color to tabanids. However, insects collected on sticky traps are not easily retrieved for identification and can be hard to count

c. Malaise trap. This trap can be used to both survey and collect live flies, but it has little advantage over collections from a bait animal and has the disadvantage of requiring specialized equipment. In trapping tabanids, trap location is extremely important. Tabanids are strong and fast flies and prefer unobstructed flyways (e.g., the border area between pastures and forested areas, roads, footpaths and trails through forested or brushy areas). Such areas tend to concentrate the flying population. In pastures, the populations are diluted by open space. Trap efficiency can be greatly increased if baited with CO_2. Use of CO_2 can increase trap efficiency by factor of 3.5 compared with unbaited traps. Trap collections should be retrieved on a regular schedule, and the intervals between retrievals should not be greater than 1 week though 1-2 days is preferable (when establishing trap intervals, factors such as number of traps, available transportation, type of terrain, and ease in reaching trap sites must be taken into consideration.

d. Canopy or Manitoba trap. This is the most effective way to collect large numbers of Tabanids, optionally baited with octenol (1-octen-3-ol), acetone or dry ice.

e. Hand-operated sweep net. Horn flies can be easily collected from penned animals with an insect sweep net. Simply using an insect sweep net to collect the flies coming to bite can monitor adult horse fly activity. This type of sampling will give qualitative information on species present in the area. From

the relative numbers of the various species collected by this means, some inference concerning the predominant species may be drawn.

5. Surveillance data and action threshold.

No action thresholds have been developed for integrated tabanid management programs. The human tolerance threshold for tabanids (horse flies and deer flies) tends to be relatively low, since their bites are painful and their “buzzing” around the head is annoying. However, control measures against larval and adult tabanids are difficult to implement and relatively ineffective. In feedlots and dairies, more than 5 stable flies per leg are considered an economic level. It is recommend spraying dairy cattle when there are more than 25 flies per animal. The economic threshold for the stable fly on cattle is, 25 flies per animal per day. Spraying beef cattle when there are more than 50 flies per animal is recommended. Researchers have recorded as many as 1000 flies per animal per day. No action thresholds have been developed for stable flies attacking humans. Thus, the decision to take action is usually based on complaints from those affected. Stable fly feeding can cause a decline in production due to the animals' fatigue from trying to dislodge the flies.

6. Preservation.

Specimens should be pinned while fresh (within 24 hours), placed in pinning boxes, and identified as noted. If this is not feasible, killed specimens may be packed in a soft material such as facial tissue. Do not use cotton because specimens will become entangled in the fibers, and there will be extensive loss of body parts needed for identification. Never place specimens in a preserving fluid such as alcohol, since this will destroy many identifying characters. Correctly packed specimens should be stored in a refrigerator or freezer (if possible) until they can be sent to a laboratory for identification. Larvae, like those of other Diptera, preserve well in 80% ethanol, but are best killed first in hot water as this helps to retain their natural shape.

CHAPTER 10: Filth Flies (order Diptera)

1. Military Importance.

Filth flies have historically had and continue to have an important impact on combat, peacetime contingency operations, disaster relief operations, and refugee health support operations. Filth flies may interfere with military operations through transmission of disease (typhoid, dysentery, and other diarrheas), contamination of food, and annoyance or distraction from the job at hand. These disease conditions constitute a significant public health hazard and have impacted military operations throughout history. Many reports of concurrent increases in fly populations and diarrheal rates come from military campaigns during World Wars I and II. In 1958, a U.S. Marine Corps force sent to Lebanon was incapacitated by dysentery within two weeks. USMC forces deployed to Lebanon in 1982-3 relied heavily upon preventive medicine for protection. This commitment to good sanitation practices and extensive fly control resulted in very low diarrheal incidence.

2. Background.

Filth flies are several species of true flies that belong primarily to the families Muscidae, Calliphoridae and Sarcophagidae (e.g., house fly (*Musca domestica*), eye fly (*Musca sorbens*), face fly (*Musca autumnalis*), lesser house fly (*Fannia canicularis*), blow fly (*Phaenicia sericata*), false stable fly (*Muscina stabulans*) and several species of *Calliphora*, *Lucilia,* and *Phormia*) are potential carriers of organisms that cause typhoid, dysentery, and other diarrheas. Recently, it was shown that common house flies carry bacteria (*Acinetobacter baumannii*) that has been linked to meningitis. In addition, *Bacillus pumilis*, which can cause food poisoning and *Enterobacter sakazakii*, which can cause urinary tract, pulmonary, and bloodstream infections, were also found. Filth flies transport disease organisms to food on their feet and body hairs. They contaminate food whey they regurgitate on the food to liquefy it for ingestion.

The Armed Forces Pest Management Board Technical Information Memorandum (TIM) No. 30, "*Filth Flies: Significance, Surveillance, and Control in Contingency Operations*", is an excellent reference document and provides additional detailed information. It is available as guidance for download from the AFPMB web site at URL **http://www.acq.osd.mil/afpmb/** which will print in 8.5" by 11" format.

Installation food handling facilities should be the primary focus of filth fly surveillance. The presence of filth flies is an indication that waste handling practices in the area are not sufficient to prevent breeding of flies. House flies can complete their development in as little as 6 days. Infrequent garbage collection will allow flies to complete their development before refuse is removed.

3. Geographic distribution and habitat.

The house fly has a worldwide distribution. The eye fly inhabits the Old World tropics and subtropics, Australia and Pacific Islands. The face fly was found only in all regions of the Old World in the past. However, now it is found throughout the Western Hemisphere since its introduction into the United States in 1952.

4. Procedures.

There are many techniques for sampling filth flies. During contingency operations, **the most appropriate methods for filth fly surveillance are counting the number of filth flies present on resting sites or those caught by sticky or bottle traps.** Conduct a preliminary survey at all potential filth fly infestation sites listed. The purpose of this survey is to identify existing fly populations. Weekly fly surveys should be conducted throughout the fly breeding season. Sampling methods used to determine fly population estimates must by the same from one survey to the next. The sampling should be conducted at a standardized time and at the same locations.

a. Fly grill technique. The fly grill technique is the most versatile and widely used of the counting techniques. The grill, often referred to as a Scudder grill, consists of 16-24 wooden slats, fastened at equal intervals to cover areas of grill 0.8 m^2 (big grill) down to 0.2 m^2 (small grill). For general use, a small or medium-sized grill is most suitable. Place the grill where there are natural fly concentrations and count the number of flies landing on the grill for a given period of time (usually 30 to 60 seconds). In each location, average the counts made on the 3 to 5 of the highest fly concentrations.

b. Fly bait technique. This technique is used to determine fly densities indoors. Place a square card 30 cm on a side that has been painted with a mixture of molasses and vinegar (1:2 ratio) near a location frequented by flies. Other baits can be used (e.g., syrup, molasses, or milk), but in order for fly counts to be meaningful, uniformity of bait and technique is necessary. Record the number of flies attracted to the card for a specific period of time (e.g., 5 minutes). The simplest monitoring method is to place 8 x 13 cm white index cards on resting sites, and count the number of fecal and vomit spots that accumulate weekly. Usually, 100 spots per card per week is an indication of high populations that require treatment, although this level will vary from site to site (Lysyk and Moon 1994).

c. Sticky tapes. Sticky tapes or strips are a useful method for assessing fly densities, particularly indoors. Sticky fly tapes hung from the ceilings over areas frequented by flies are effective in surveying populations of house flies. The sticky tapes or stripes may be exposed to flies from 2 hours to 2 days (1

day is recommended). However, the usefulness of the sticky tape is limited to 1-2 days because the sticky surface soon loses its fly-holding ability. For best results, the tape should be replaced daily, and the flies trapped on the tape exposed the previous 24 hours can be counted. The length to time and time of day must be consistent for the data to be meaningful. The strips should be located near doorways or trash receptacles. However, they should NOT be placed over food preparation or serving areas. It is recommended using 4 tapes per 10,000-ft^3 floor space with a minimum of 5 feet between tapes. Totals of 50-75 assorted flies (accurate identification of the glue covered flies is often difficult) per tape per day indicates a heavy fly population.

e. Live traps. These are recommended only when live specimens are required for identification or resistance testing. They provide quantitative data for fly surveillance, but are not as convenient as sticky tapes or Scudder grills for routine surveillance. Empty plastic water or soft drink bottles may be modified for use as traps.

f. Baited jug-traps. These traps can also be used to monitor fly populations. These are plastic pails with 3.5 cm holes cut in the sides near the top. The pails are hung indoors from rafters, and baited with 25 g of house fly bait that includes a toxicant and chemical attractant specific for house flies (Burg and Axtell 1984). The number of flies captured per unit time is used as a measure of abundance, and 300 flies per trap week is a common treatment threshold (Lysyk and Moon 1994).

g. Hand-operated sweep net. The sampling technique is useful in obtaining flies for identification, but quantitative estimates of the fly population are difficult to obtain and unreliable. Collection of face flies, a common pest of cattle, is best achieved by passing an insect net over the face and shoulders of fly-infested animals.

h. Visual counting. This survey technique is recommended for the face fly because it is not responsive to baited or lighted traps as the house fly and is active only during the hours of bright daylight. Therefore, passive survey methods are ineffective, and visual counts of flies on cattle is recommended. Count only the flies on the faces of animals and make fly counts on at least 15 animals in each herd. More than 10 flies per face is indication of a high fly population. Visual counting can also be used as a “field expedient” method for counting other filth flies. Flies can be counted on any given surface, such as a table top, tent pole, appliance, or even a person in the area.

5. Surveillance data and action threshold.

Records of filth flies trapped and counted are useful indices monitoring fluctuations in fly populations. A composite index for a particular area or installation is calculated by averaging data from several collection sites. The

composite indices should be plotted in graph form to visualize population levels. A threshold can be established after surveillance data is collected over a period of time. Example: a grill index of 25 flies in residential areas is usually considered unacceptable; for baited jug traps, the number of flies captured per unit time is used as a measure of abundance, and 300 flies per trap week is a common treatment threshold.

6. Preservation.

Specimens should be pinned while fresh (within 24 hours), placed in pinning boxes, and identified as noted. If this is not feasible, killed specimens may be packed in a soft material such as facial tissue. Do not use cotton because specimens will become entangled in the fibers, and there will be extensive loss of body parts needed for identification. Never place specimens in a preserving fluid such as alcohol, since this will destroy many identifying characters. Correctly packed specimens should be stored in a refrigerator or freezer (if possible) until they can be sent to a laboratory for identification. Larvae, like those of other Diptera, preserve well in 80% ethanol, but are best killed first in hot water as this helps to retain their natural shape.

CHAPTER 11: Myiasis-Producing Flies

1. Military Importance.

Myiasis refers to the condition of fly maggots infesting the organs and tissues of people or animals. Myiasis is rarely fatal, but troops living in the field during combat are at high risk of infestation. Extra care should be taken to keep all wounds clean and dressed. Although myiasis tends to be an uncommon event affecting single individuals, the threat of this condition can produce significant psychological anxiety among military personnel.

2. Background.

Flies producing myiasis (an infestation of human and vertebrate animals with dipterous larvae) are either obligatory parasites (those species whose larvae develop exclusively in living vertebrates) or facultative parasites (those species whose larvae are normally free living and develop in decaying organic matter, but may be able to develop in a living body). The important myiasis-producing Diptera are grouped taxonomically in five families: Gasterophilidae, Oestridae, Cuterebridae, Calliphoridae, Sarcophagidae, and Muscidae.

3. Geographic distribution and habitat.

The Old World screwworm (*Chrysomya bezziana*) is found throughout the Afrotropical and Oriental regions extending south into Indonesia, the Philippines and New Guinea. The New World screwworm (*Cochliomyia hominivorax*) is found only in South and Central America. The black blow fly (*Phormia regina*) is common in northern latitudes worldwide and as far south as Mexico. Calliphora spp. are most common in the northern parts of the Old and New Worlds and in the Australian region, although two species, *C. croceipalpis* and *C. vicina*, occur in the Afrotropics. *Dermatobia hominis* commonly attacks humans in Latin America, the larvae of which are carried to people on phoretic hosts, especially mosquitoes of the genus *Psorophora*.

4. Procedures.

To collect larvae and adults of myiasis-producing Diptera, especially the obligate parasites, one must know where and when to search animals and the environment for them.

a. Hand-operated sweep net. Usually the adults of facultative parasites are fairly abundant in nature and can be collected with a sweep net in the area near the dead and decaying animal matter to which they are attracted. The sampling technique is useful in obtaining flies for identification, but quantitative estimates of the fly population are difficult to obtain and unreliable.

b. Visual counting. The adults of a few species, including cattle grubs, may be found (with careful study) hovering around hosts or at a variety of aggregation sites. However, this surveillance method is time-consuming and requires a considerable amount of taxonomic skill on the part of the person conducting the surveillance. Calliphorid and sarcophagid larvae found on or in the skin of animals can be collected by removing them with forceps. Because of their facultative nature, they are usually abundant. They can be reared to adults, if necessary.

5. Surveillance data and action threshold.

Little information is available concerning the rating of infestations of myiasis-producing Diptera. However, the following rating system for infestations of certain obligatory myiasis-producing Diptera in domestic animals may be a useful guideline to follow:

Number of pests/area				
Pest	*How rated*	*Light*	*Moderate*	*Heavy*
Horse bots	Eggs/square inch	<2	2-5	>5
	Larvae/gastrointestinal tract	<10	10-50	>50
Sheep nose bots	Larvae/head	<10	10-25	>25
Cattle grubs	Larvae/animal's back	<5	5-15	>15

6. Preservation.

Because of the limited and fragmentary information concerning the taxonomy and identification of immature forms of myiasis-producing Diptera, larvae that are collected should be reared, if possible, to the adult stage for identification. The larvae of facultative parasites found on carcasses may be removed from the animal's body and placed on a piece of decaying meat. The meat is then placed over dry sand in a closed container to provide a place for pupation of fully-grown larvae and to prevent escape of larvae or the invasion of unwanted larvae onto the meat. When the larvae have finished feeding, they will crawl out of the meat medium and pupate in the dry sand. Adults that emerge from pupae should be fed a diet of honey water (or sugar water) until the cuticle has dried (allow 1-2 days). The adults can be killed in standard, insect-killing jars or by exposure to freezing temperatures, and properly pinned for identification and/or shipment. The pinned and dried adults tend to be fragile, therefore, special care should be exercised in packing for shipment to experts for identification.

Larvae of obligate parasites can only fully develop within the host. Some larvae can be expressed from the dermal cysts by gentle pressure around the breathing pore of the larva, but others live deep inside the skin and can be obtained only by surgical methods. If larvae are collected, the best way to kill them is to place them in hot water just below the boiling point; the heat causes

the cuticle to expand, so these larvae die in an extended state with which taxonomists can work more easily. If the water is actually boiling the larvae will tend to rupture. These larvae should then be placed in 70-80% ethanol with no additives.

The importance of proper labeling cannot be over-emphasized. A specimen is of little or no value if it is without a label giving details of where and when it was collected. The label should state the origin of the myiasis case, location, nature of the wound, and date.

CHAPTER 12: Fleas (Order Siphonaptera)

1. Military Importance.

Only of few flea species are directly associated with disease transmission to humans and these are normally limited to those having direct access to both human and wild animal populations. The fleas of chief interest are those, which are commonly found on rats and other rodents subject to plague and endemic typhus, and those, which attack humans. Human intrusion into an area where the wild host population is under the stress of an epizootic vastly increases the chances of acquiring the disease, even if the fleas are poor vectors to humans. During military operations, fleas may be encountered in large numbers shortly after entering an abandoned building. When a building is abandoned, flea pupae will remain in a quiescent state for long periods of time. The activity of anyone entering such premises will stimulate a mass emergence of hungry fleas.

2. Background.

Fleas can affect human health in several ways: as biting pests, burrowing "jiggers", parasite intermediate hosts and vectors of pathogens. When a flea inserts its mouthparts into the skin of the host, saliva in injected and blood is pumped upwards. The saliva prevents coagulation of the blood and is the cause of the skin reaction, manifested as erythema and edema. *Tunga penetrans* (occurring in Central and South America, in tropical Africa, and in India) is the only species of the so-called jigger, chigoes or sand-fleas that has medical importance. Several parasitic worms are associated with fleas. One of the most common is the double-pored tapeworm (*Dipylidium caninum*) of dogs and cats, which can be an occasional human parasite. Fleas transmit protozoans, bacteria, rickettsia and viruses between mammals but the main diseases transmitted to man are bacterial and rickettsial. Fleas can transmit the following diseases to man: plague (*Yersinia pestis*); tularemia (*Francisella tularensis*) isolated from fleas in Scandinavia, Russia and USA; pseudotuberculosis (*Yersinia pseudotuberculosis*) isolated in far eastern Russia; and erysipeloid (*Erisipelothrix rhusipopathiae*), which occurs only in Asiatic Russia. In addition, fleas are confirmed vectors of the rickettsial infection murine typhus (*Rickettsia typhi*), a worldwide infection of murine rodents that in man is clinically milder than the louse-borne typhus.

3. Geographic distribution and habitat.

Fleas occur worldwide, and are found in such inhospitable locations as the Arctic and Antarctic. Fleas occur from sea level to high altitudes wherever satisfactory hosts occur. While the order Siphonaptera is worldwide, some of the families are quite restricted in their occurrence and only a few are cosmopolitan.

Fleas are small, wingless insects that are laterally compressed, with numerous bristles and appear shiny yellowish-brown to black. Adult fleas are obligate bloodsucking parasites of warm-blooded vertebrates and 94% of known species occur on mammals, the remaining 6% feed on birds. The larvae are not normally parasitic, but rather feed on organic material in the nest or hair of the host. Larval flea survival is >90% at temperatures of 21-32^{0}C, but survival drops to <40% at 38^{0}C. In addition, relative humidity's of <45% or >95% results in also no larval survival. The length of the life cycle of the flea ranges from 1 month to over a year, depending upon environmental conditions.

4. Procedures.

Collection of fleas can be made from either the host or the environment, depending on the species and the situation. However, as with other groups of ectoparasitic arthropods, host animals are the primary source of most flea specimens. Climatic conditions are very important. In general, fleas are never as abundant during the winter and spring as they are in the summer and fall, but there are exceptions. For example, in the southern US, the human, dog, and sticktight fleas are abundant in late spring and early summer when rains are frequent and humidity is high. The presence or absence of hosts has a direct effect on the numbers of certain fleas.

a. Animal host trapping. Hosts can be captured alive and anesthetized briefly while they are brushed or vacuumed for parasites. Live traps should be used as the fleas will quickly leave the host's body if it is killed in a standard break-back or "snap" mousetrap. When collecting hosts, care must be taken to place each host animal in a separate container to prevent parasite transfer from one host to another. Small plastic bags can be used, but cloth bags are preferable since they soak up body fluids and can be washed when necessary. Bags can be used in a large can or other closed container and sprinkled with chloroform to immobilize the ectoparasites. Each host is removed from its bag while the bag is examined for parasites that have left the body of the host. The host body is then brushed, combed or tapped against the surface until ectoparasites cease to appear. Ears, nostrils, the mouth and other body openings should be carefully examined for fleas that may have entered in an attempt to escape the fumigant. Exposed parts of the host such as ears, nose, feet and tail should be examined for fleas that may be attached by their mouthparts or embedded in the host's tissues.

b. Examination of nest material. Nesting material taken from the lair, or from guano piles beneath bat roosts, can yield large numbers of adult fleas if kept in paper bags or other containers in the laboratory. Samples should be kept at room temperature and sprinkled with water periodically to keep them moist but not wet. They should be checked for adults every few days until adults cease to emerge.

c. "White-sock" technique. Collect and count fleas landing on the observer's lower legs using the "white-sock technique" (the observers wear white socks pulled over light colored slacks).

d. Attraction to a light source. Adult fleas can be collected from dwellings by placing a small kerosene or oil lamp over or candle a pan of soapy water. Fleas attracted to the flickering light are trapped when they jump into the soapy water.

e. Vacuuming. Vacuum sample (using a portable vacuum cleaner with a handkerchief inserted in the dust collection bag) and count the fleas collected.

5. Surveillance data and action threshold.

In assessing flea populations, the relationship of host density to flea density should be considered. It is common practice to use a flea index (average number of fleas per host). The flea index, though it is often used for rodent fleas, may not always be the most accurate measurement of a population. Usually only adults are considered and then only while they are on the host for feeding.

In addition, an "absolute flea index" (AFI), (the average number of fleas, including nest fleas, that theoretically can feed on one host) has been suggested. It is derived from the following formula:

$$AFI = \frac{RF + NF}{TR}$$

When RF = the flea population on rodents
NF = the flea population in nests and burrows
TR = the total rodent population

This index is intended for use on rat fleas, and is admittedly difficult to obtain. Nevertheless, it is suggested as a goal whenever feasible. For the Oriental rat flea (*Xenopsylla cheopis)*, a flea index of > 1.0 flea per host is considered high. Different indices have been developed for flea action thresholds: i.e., Indoors, 5 or more fleas on the legs of observers in < 1 minute is indicative of a severe infestation. Five or more fleas on a pet indicate a severe infestation. Fleas in homes may be considered to be above the threshold level when they cause bites on the residents. In plague endemic areas, bivouacking and other outdoor activities should be restricted when fleas are at an outbreak level.

6. Preservation.

Fleas can be stored both, temporarily or permanently in 75-80% ethanol. However, it is also acceptable to store fleas dry in vials and held in place with non-absorbent cotton or tissue. All specimens from a single host should be kept

together in the same container during processing. For most fleas, accurate identification requires that they be cleared and mounted on microscope slides.

CHAPTER 13: Sucking Lice (Order Anoplura)

1. Military Importance.

Military historians generally agree that the louse-borne diseases have killed more soldiers than the bullets fired during conflict. In one of the worst disasters in military history, over one-half of Napoleon's army perished from epidemic typhus during the invasion of Russia in 1812. More recently, epidemic typhus outbreaks during World Wars I and II, killed thousands of civilians. However, U.S. Army personnel experienced on 30 cases of typhus with no typhus deaths in the North African-Middle East-Mediterranean zone during the years 1942-1945. This was attributable to good preventive medicine measures.

Heavy and persistent infestations with lice can cause insomnia, nervousness, anemia, and general loss of condition, as well as dermatitis, bleeding, and scab formation enhanced by scratching. However, the real medical importance of lice is principally in the life-threatening diseases the body louse transmits: classical epidemic typhus (*Rickettsia prowazeki*), louse-borne relapsing fever (*Borrelia recurrentis*) and trench fever (*Bartonella quintana*). Today, these diseases are confined to generally impoverished and often disaster-struck regions of the world. They occur in all continents other than Australia and Antarctica, but are particularly significant in Africa. Mortality due to epidemic typhus under war conditions is 5-25 percent and can reach levels of 50-70 percent.

2. Background.

Lice are parasitic insects that spend their entire life cycle on the host and never voluntarily except to transfer to a new host. Both sexes of the adult and all nymphal stages suck blood. They are highly host specific. Humans are parasitized by three species of lice, all members of the order Anoplura (sucking lice). There are two genera, *Pediculus*, containing *P. humanus humanus* (the body or clothing louse), *P. h. capitis* (the human head louse) and, and *Pthirus* with a single species *Pthirus pubis* (the pubic louse).

3. Geographic distribution and habitat.

The three human lice species are distributed worldwide. Generally, *P. h. humanus* is limited to sectors of the population that are not able to change their clothes regularly, usually because they possess only one set (e.g., refugees or displaced persons). Each species of human louse specializes in infesting particular parts of the body. The head louse (*Pediculus capitis*) occurs in head hair and cements it eggs on head hair shafts whereas the closely related body louse (*Pediculus humanus*) lays its eggs on clothing fibers and visits the body to feed. *Pthirus pubis* is found primarily on pubic hair, but can be found on any coarse body hair, including the eyelashes, facial hair and occasionally on head hair.

4. Procedures.

The collection and surveillance for sucking lice depends primarily on meticulous examination of human hair for head lice or clothing, particularly the underwear, for body lice to detect egg, immature and adult specimens. Immature forms are often found actively feeding on the skin under the hair. For detecting head lice, conduct a 3-5 minute examination of each person looking for nits (eggs) within ¼ inch of the scalp and crawling lice (nymphs or adults). An estimate of intensity of louse infestation can be made by counting the number of crawling lice observed in 2 minutes within ¼ inch of the scalp. Use forceps or a fine brush moistened with alcohol to remove the lice from the hairs of the host. Drop the specimens into small vials of ethanol, using a separate vial for each species and for specimens from different hosts. When epidemic typhus and body lice are involved, those examining people for infestation should protect themselves from infection by inhalation of infective louse feces.

5. Surveillance data and action threshold.

Populations of all three species of human lice may sometimes reach very high levels, thousands (1,000 – 10,000) being recorded from single hosts, but generally lice number no more than about 10 per person in infected communities. The presence of any lice should prompt immediate action (e.g., **the action threshold is one louse**). An important first step in the process is public education. The biology and control of the louse species involved in the infestation should be explained to the persons affected and their cooperation should be sought in managing the problem. For example, in a school situation, easy-to-understand information should be provided to the children, parents, teachers and administrators. In this type of situation, the local public health department would likely handle the public information program.

The Armed Forces Pest Management Board Technical Information Memorandum No. 6, *Delousing Procedures for the Control of Louse-borne Disease During Contingency Operations,* is an excellent reference document and provides additional detailed information on body louse control.

6. Preservation.

Lice can be preserved in 75% ethanol. However ordinary 70% rubbing (isopropyl) alcohol) or 10% formalin solution would be acceptable if nothing else is available. Some lice can be kept alive for several days in a dry container held at ordinary temperatures while awaiting shipment to a laboratory for disease pathogen testing.

CHAPTER 14: Bedbugs and Kissing-Bugs (bloodsucking Hemiptera)

1. Military Importance.

The Triatominae (commonly known as “kissing bugs” or “cone-nosed bugs”) are renowned vectors of *Trypanosoma cruzi*, causative agent of Chagas disease or South American trypanosomiasis. The disease is believed to affect 16-18 million people, primarily in Central and South America; an estimated 100 million people are at risk, accounting for approximately 25% of the entire population of this region. Chagas disease accounts for an estimated 45,000 deaths each year and is ranked third behind malaria and schistosomiasis by WHO in terms of global burden as a tropical disease. Since soldiers may operate in the forest, it is more likely they will be bitten by kissing bugs that live outside domestic building (extradomiciliary), especially if they do not use personal protective measures. In addition, the disease has the potential to infect troops that bivouac (sleep) adjacent to or in areas with substandard housing (e.g., makeshift and palm thatched construction). Avoiding such areas and using personal protective measures to prevent biting bugs will minimize risk. Because serious symptoms are delayed, the greatest impact will be on the individual service member and the medical support system long after redeployment.

2. Background.

The Hemiptera are most easily recognized by their general body form and by their piercing and sucking mouthparts. The Family Cimicidae (bedbugs) and Family Reduviidae (assassin bugs), Subfamily Triatominae belong to the Order Hemiptera or “true bugs”. These are exclusively blood-feeding insects.

Bedbugs are small, virtually wingless insects living close to their vertebrate hosts. There are two species that principally feed on humans, *Cimex lectularius* and *C. hemipterus*. These are primarily of medical importance as nuisance insects that can contribute to chronic iron deficiency anemia. However, they may have a minor role in the transmission of some blood-borne viruses such as hepatitis-B. Adults are about 5 mm long and 3 mm broad. Their color ranges from yellowish to dark brown but often appears dark red when the bugs are recently fed. All five nymphal stages and both sexes of adults are obligate feeders on vertebrate blood.

The greatest importance of the Subfamily Triatominae is their capacity to transmit *Trypanosoma cruzi*, causative agent of Chagas disease or South American trypanosomiasis. Epidemiologically, only about a dozen species have become sufficiently closely associated with humans to represent a public health problem. The most important vector species are *Triatoma infestans*, *T. brasiliensis*, *T. dimidiata*, *Panstrongylus megistus*, and *Rhodnius prolixu*s. Domestic infestations of triatomine bugs can be very stressful, especially where the occupants are unable to afford remedial action. All triatomine vector species

are obligate bloodsuckers. Domestic bug populations typically reach several hundred individuals of different stages, with each bug feeding every 4 - 9 days. This translates to biting rates often in excess of 20 bites per person per night, representing a daily blood loss averaging 1 – 3 ml per person.

3. Geographic distribution and habitat.

Cimex lectularius (the common bedbug) is a widespread household pest in most parts of the world. Of the118 known species of Triatominae 115 occur in the New World, roughly from the Great Lakes region in the USA to southern Argentina (i.e., from latitude 42^0N to 46^0S). A single species, *Triatoma rubrofasciata*, is distributed world wide, wherever *Rattus norvegicus* occurs. Fortunately, this species is not a vector of *T. cruzi*.

Bed bugs are mostly active at night and are extremely shy and wary; thus their infestations are not easily located. Bed bugs normally feed at night while the victim is lying down and sleeping, and thus their bites may be on the face, neck, arms, or hands. Infestations of bed bugs indicate their presence by leaving blood stain smears on walls, bedding, curtains, and other light-colored surfaces. Bed bugs nymphs and adults can survive over a year without feeding (especially at temperatures below 18^0C), reappearing from their crevices when hosts become available. Female bedbugs commonly lay their eggs in cracks in crevices of houses (e.g., behind torn wallpaper) and outbuildings. Heavily infested premises can generally be recognized by fecal spots on walls and furniture, and by the characteristic “buggy” odor emitted from adult metasternal scent glands. It is reminiscent of the aroma of the spice coriander or fresh red raspberries.

Triatomine bugs are found in almost any habitat offering a degree of permanence, climatic shelter and ready access to a blood source. All nymphal stages and both sexes of adults are obligate feeders on vertebrate blood, and usually occupy the same habitat. Most species of Triatominae tolerate a range of atmospheric humidity between 30 – 80%. Most species thrive at temperatures between 24 –28^0C. Development is usually halted at temperatures below 16^0C, while temperatures above 40^0C are usually lethal. In rural houses in Latin America bug populations numbering in the thousands have been recorded.

4. Procedures.

Basic information on the preferred ecotopes of each target bug species is crucial for effective surveillance. Domestic and peridomestic structures housing humans and domestic animals are important sources of cimicids and triatomines. Detecting triatomine and cimicid infestations is a difficult task when bug densities are low. Quantitative sampling of bugs in houses is difficult and subject to extreme bias in favor of large stages. Baited traps are ineffective.

a. Direct observation with or without a flushing agent. Domestic and peridomestic Triatominae are normally collected using long, blunt-ended forceps, and a flashlight to illuminate cracks where the bugs may be hiding. Irritant sprays (flushing-out agents) are sometimes used to encourage bugs to leave cracks and facilitate their capture. Timed manual capture (0.5 person-hours per compound) using a flushing-out spray and flashlight (FO method) is usually considered the method of preference for assessing domestic (mattresses, bed frames, posters, and wall cleft) and peridomestic (piles of tiles, firewood, inside chicken-houses, and near cattle) bug habitats. These sprays are generally prepared from dilute suspensions of various pyrethroid insecticides (e.g., d-Phenothrin). Domestic cimicids can be collected in much the same way. Bugs can also be knocked down by controlled insecticide application (KD method). The FO method is more sensitive, but the KD method is faster and better suited for standardization.

b. Observations using sheets of paper. Sheets of paper are tacked to bedroom walls for long periods and reveal infestations through the presence of triatomine fecal streaks, exuviae, eggs or bugs. At the beginning of an infestation in a bedroom, bed bugs are likely to be found only on the beds. At first they are apt to hide about the tufts, seams, and folds of mattresses and daybed covers; later, they spread to cracks and crevices near the beds.

5. Surveillance data and action threshold.

Seriously bed bug infected homes and apartments often can be recognized by their distinctive “buggy” smell, an odor that has been described as “obnoxious sweetness”. This odor also has been likened to that of fresh red raspberries. Because bed bugs bite, produce an obnoxious odor, and are considered a sign of poor sanitary conditions, the presence of any bed bugs in a home or facility is considered adequate reason to begin a control program.

6. Preservation.

Both immature and adult kissing bugs can be killed and preserved in 75% ethanol. However, for permanent preservation, adults should be pinned. Specimens too small or too slender for direct pinning should be glued to points on double mounts. Bed bugs should be preserved in 70% ethanol. For collection and identification, bugs can be killed with any standard killing agent (e.g., ethyl acetate or chloroform) or heated (e.g., in tropical sunlight) to above 45^0C for 10 – 20 minutes.

CHAPTER 15: Cockroaches (Suborder Blattaria)

1. Military Importance.

Because of their filthy habits, cockroaches are potential vectors of human disease. They visit sewers, privies, septic tanks, cesspools, dead animals, bloody bandages, pus and many other contaminated materials. In turn, they visit human food, dishes, cooking utensils and other food-contact surfaces. It is probable the cockroaches rival flies as mechanical carriers of infectious agents.

2. Background.

Like flies, cockroaches have been associated with mechanical transmission of a vast number of pathogens. Bacterial agents of disease include (*Salmonella* spp., *Shigella* sp., *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Campylobacter jejuni, Serratia sp*., and *Clostridium perfringens*), systemic infections (*Toxoplasma, Streptococcus pyrogenes, Mycobacterium leprae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), and skin infections (*Staphylococcus* spp. and *Proteus* spp.). Cockroaches can also transmit the fungus *Asperigillis fumigatis* and the helminthes *Ancylostoma duodenale* (hookworm), *Ascaris* spp. (roundworm), *Enterobius vermicularis* (pinworm), *Hymenolepis* sp. (tapeworm), *Necator americanus* (hookworm), and *Trichuris trichuria* (whipworm), molds, protozoans, and viruses. In addition, cockroaches contaminate food, imparting an unpleasant odor and taste. Inhaled and ingested allergens associated with cockroaches (“cockroach asthma”) are well known.

The pest cockroaches most likely to be encountered indoors, may be grouped as follows: common pests, nearly always associated with humans, *Blatta orientalis* (oriental cockroach), *Blatella germanica* (German cockroach), and *Periplaneta americana* (American cockroach); less common, but frequently associated with humans, *Supella longipalpa* (brown-banded cockroach), *Periplaneta australasiae* (Australian cockroach), *P. brunnea* (brown cockroach), *P. fuliginosa* (smoky brown cockroach), *Leucophaea maderae* (Madeira cockroach); wild species which occasionally invade buildings or occur in goods, *Pychoscelus surinamensis* (Surinam cockroach), *Nauphoeta cinerea* (lobster cockroach), *Blaberus* spp. and about ten other species.

3. Geographic distribution and habitat.

Many species of cockroaches have become cosmopolitan and, in particular, some which have penetrated cool climates by living almost exclusively inside warm human habitations. During the daytime, cockroaches mainly rest in suitable harborages. Concealment, temperature, access to moisture and gregarious instincts are all factors that influence their choice of resting place. Like other invasive indoor pests, cockroaches tend to avoid daylight; also they

are prone to crawl into small crevices. *B. germanica* and *P. americanus* chose temperatures in the range 25-33^{0}C; while *B. orientalis* prefers a lower range, about 20-29^{0}C. Cockroaches need regular access to water, especially if temperature is high. Cockroaches are commonly found in groups. This is due to finding the same favorable habitat and the existence of an aggregating pheromone, which they give off. Cockroaches are omnivorous and will ingest many types of materials. These include human food, animal and human feces, various kinds of refuse, book bindings, cardboard, pastes or glues.

4. Procedures.

A thorough inspection of infested or suspected buildings should be conducted. The best time to conduct surveillance is during the earlier part of the night, at least ½ hour after lights have been turned off. Nighttime inspections also allow you to observe lapses in proper sanitation that may be contributing to the cockroach infestation.

a. Monitoring traps.

(1) Sticky Traps. The use of these simple traps can provide good evidence of the nature and distribution of an infestation both indoors and outdoors. When compared to other survey methods, these traps provide a more realistic estimate of population size for most peridomestic cockroaches. Commercially available sticky traps or rodent glue boards are the types most often used. However, traps can be formed of cardboard, folded into open-ended prisms (12.5 cm x 7.0 cm x 4.5 cm) with strips of plasticized gum along the inside base. Most of these sticky traps are baited with attractive food substances and some newer models now incorporate cockroach pheromones. In a dining facility kitchen, a minimum of 10-15 sticky traps should be used.

(2) Jar Traps. Simple traps can be made from wide mouth jars baited with a small about of pretzels or pelletized pet food that has been soaked in beer. The bait is placed in the bottom of the jar and the upper inside surface of the jar is coated with a Vaseline/mineral oil mixture to prevent cockroaches that fall into the jar from escaping. The jars have to be either placed in a freezer or CO2 used to anesthetize the cockroaches to remove them. Alternatively, the bait can be floated in a small tin foil boat on mineral or cooking oil placed in the bottom of the jar. Cockroaches entering the jar will be trapped and drown in the oil. This removes the problem of removing and killing live cockroaches from the jar trap. A minimum of 6 jar traps should be used in a dining facility kitchen.

b. Visual inspection. For visual inspections by day, a good flashlight, a mirror to reflect light back into cracks, and a willingness to climb high and low, inside and outside, are essential to determine the extent and location of cockroach infestations. Nighttime surveys conducted with a red lens flashlight, because they are done when cockroaches are most active, give a better

indication of the cockroach population. Identify all stages of cockroaches (egg cases, nymphs, and adults) during the survey and record the findings.

c. Flushing agents. Pyrethrins or other flushing agents can be used to chase cockroaches from infested machinery or other difficult-to-access harborages. In many cases, it is impossible to physically see into some insect harborages (e.g., hollow legs of tables, light sockets, cracks and crevices, and cabinet and wall voids. Only by using a flushing agent can it be determined if insects are present in these types of harborages. However, flushing agents may scatter and repel cockroaches from preferred harborages to less preferable, hard-to-reach locations or nearby uninfested premises. These effects should be considered prior to using flushing agents.

d. Vacuuming. Vacuuming can be used to survey for and physically control cockroaches. Vacuuming is very efficient and effective, particularly when used in conjunction with a flushing agent to move cockroaches from deep harborage. The vacuum must: 1) be small enough to be maneuvered easily (e.g., backpack device); (2) be able to withstand accidental suction of water, which is a distinct possibility in large kitchens; and (3) have a High Efficiency Particulate Air (HEPA) filter. Without a HEPA filter, pathogens and cockroach exoskeletons, feces, and other allergens will be removed from protected sites and become airborne, causing potential health problems for residents and workers.

5. Surveillance data and action threshold.

Surveillance data should be used to focus pesticide applications in the infested area of the facility. In most cases, no single tool will provide complete suppression of cockroach populations. However, improvements in cockroach bait formulations have made them the chemical control of choice. The key to successful baiting is using sufficient bait for the population. Therefore, be sure to follow the baiting density guide on the label. Information must be constantly evaluated and decisions made based on pest species, the specific site and extent of the infestation, available chemical and non-chemical methods, cost effectiveness, and size of area to be treated. A hand magnifying lens is a valuable tool for the field identification of cockroaches.

6. Preservation.

Nymphal and adult cockroaches should be pinned soon after they are collected and killed.

CHAPTER 16: Stored Products Pests

1. Military Importance.

Stored products pests are important pest insects on military installations resulting in significant economic losses. These pests are usually not a major problem on military deployments for operational rations due to the fact that ration storage times are usually short. However, they can become a problem if rations arrive in theater that are already infested. In addition, military preventive medicine personnel are often called up to inspect humanitarian rations and donated food during peacekeeping, peace support, and humanitarian assistance operations. These rations can be a serious problem because shipments of grain and cereals, especially wheat, are particular vulnerable to insect infestation. In order to prevent insect damage in food stores, it is important to be alert for the first signs of a pest infestation before significant economic loss occurs.

2. Background.

The great majority of insect pests of stored products belong to two orders: the Coleoptera (or beetles) and the Lepidoptera (or moths). Stored products pests destroy a wide variety of products derived from or composed of material of plant and animal origin. Most damage occurs in storage. These insects destroy by contamination much more than they actually eat. The most extensive losses are due to attack of grains and cereal products by a large number of moths, weevils, and beetles. Other pests destroy beans and peas, and still others infest dried fruits, nutmeats, spices, seeds, tobacco, and candy. Various moths and beetles destroy unprotected products made in whole or in part of hides, wool, mohair, fur, hair, feathers, down, and animal bristles. Both larval and adult beetles feed on stored products pests. However, the larvae generally cause more damage due to their voracious feeding and shedding of exoskeleton during molting. Moth larvae cause considerable damage to stored goods by feeding or by contamination of with their own products (e.g., webbing and frass). Adult moths are not responsible for damage as they either feed on liquid food and water or do not feed at all. The most important stored products pests and their preferred diets are list in the table on the next page.

3. Geographic distribution and habitat.

Most pests of stored products are of tropical and subtropical origin. However, most species of stored products pests have been distributed nearly worldwide because of modern, rapid transportation. Consequently, no areas of the world are immune to these insects. Almost all species are adapted to living on foods with very low moisture content. Very few stored products pests can live and breed at temperatures above 95^0F, and most do not lay eggs at temperatures below about 60^0F. Nearly all insect stored products pests have been recorded from a wide variety of stored foods, spices and drugs. Nevertheless, many

pests are typically associated with a particular type of product as listed in Table 16-1, below.

Table 16-1. Major Stored Products Pests of Worldwide Importance

Major Pests Of Grains And Cereal Products		
Common Name	**Scientific Name**	**Products Attacked**
Angoumois grain moth	*Sitotroga cerealella*	Whole grains (such as wheat, barley, rye, corn, oats, rice, and various seeds)
Indian meal moth	*Plodia interpunctella*	Grains and cereal products
Mediterranean flour moth	*Anagasta kuehniella*	Flour and meal
Rice weevil	*Sitophilus oryza*	Whole grains
Granary weevil	*Sitophilus granarius*	Whole grains
Lesser grain borer	*Rhyzopertha dominica*	Whole grains and flour
Saw-toothed grain beetle	*Oryzaephilus surinamenis*	Whole grains, flour, and meal
Confused flour beetle	*Tribolium confusum*	Cereal products such as flour and meal
Cadelle	*Tenebroides mauritanicus*	Whole grains, flour, and meal
Yellow mealworm	*Tenebrio molitor*	Meal, flour, bran, cereals, bread, and crackers
Khapra beetle	*Trogoderma granarium*	Wide variety of grains, flours and cereals
Major Pests Of Legumes, Dried Fruits, And Vegetables		
Common bean weevil	*Acanthoscelides obtectus*	Beans in storage
Cigarette beetle	*Lasioderma serricorne*	Tobacco products, cotton seed meal, dry yeast, spices, dried fruits, grains, cereal, seeds, dried meats, leather, and fabrics
Tobacco moth	*Ephestia elutella*	Tobacco products, chocolate, grains, seeds, cereal, peas, beans, peanut meal, coffee, biscuits, and spices
Major Pest Of Foods Of Animal Origin		
Redlegged ham beetle	*Necrobia rufipes*	Ham and other dried meats, bacon, fish, coconuts, biscuits, cheese and garlic
Larder beetle	*Dermestes lardarius*	Ham and other dried meats, bacon, fish, cheese, tobacco and biscuits
Cheese (ham) skipper	*Piophila casei*	Cheese, cured meat (ham and bacon) and fresh meat (beef-steak)
Major Pests Of Fibers And Fabrics Of Animal Origin		
Black carpet beetle	*Attagenus megatoma*	Clothing and furnishings containing wool, hair, and other animal products
Common carpet beetle	*Anthrenus scrophulariae*	Carpeting and furnishings containing wool, hair, and other animal products
Webbing clothes moth	*Tineola bisselliella*	Wool, hair, and fur
Case making clothes moth	*Tinea pellionella*	Clothing, furnishings, wool, hair, bristles, felts, feathers, hides, tobacco, drugs, spices and nut meats

4. Procedures.

a. Visual inspection of storage area. Note the number and type of dead insects on floors, ledges, and windowsills, as well as any live insects crawling on or flying around commodities.

b. Commodity inspection. Check infestible food products by moving a layer or two of packages off the top of the pallet and look closely for insects on package surfaces or in cracks and folds between packages. Insects infesting food products will usually be found on the underside of plastic bags, in and along folds and seams of bagged commodities, in the bottom of cartons and sacks, or underneath carton or box flaps. The existence of "frass" or silk in or on a product or chewed entry or exit holes are clues to an infestation, even if actual insects are not immediately seen. Using a flashlight is highly recommended as food storage warehouses usually have poor lighting.

Inspecting operational rations during deployments deserves special mention here. It is extremely important to ensure that all operational rations remain pest free during deployments. Conduct through veterinary/entomological commodity inspections for all possible infestations during receipt of goods and on a minimum, monthly basis. Reported problems and deficiencies must be closely monitored until corrected. To conduct a proper inspection, the inspector needs a flashlight, pocket knife for opening boxes, clipboard, paper, pen, and tape for resealing boxes. In addition, the inspector may want to carry chalk for marking infested pallets, a magnifying hand lens to aid in initial identification, and a mirror on an extendable handle for inspecting difficult to reach locations. Also, a portable black light is useful in determining rodent contamination, as rodent urine fluoresces under ultraviolet light (see Chapter 21). DO NOT place infested goods in the warehouse. Immediately report all Meal, Ready-to-eat (MRE) shipments showing evidence of infestation to Defense Supply Center, Philadelphia (DSCP) for pest management guidance. In addition to inspecting MREs, all other highly infestible commodities (dry pet food, flour and bakery mixes, nuts and dry fruit, pasta, candy, grits, cookies, cornmeal, dry beans, cereals, spices and rice) should be subject to monthly inspections. More information is available in AFPMB Technical Information Memorandum No. 38, *Protecting Meal, Ready-To-Eat Rations (MREs) and Other Subsistence During Storage* and downloadable guidance available at URL **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format.

c. Light trap. A New Jersey or SSAM light trap can be used to monitor certain flying, stored products pests. Light traps should be placed at least 50 feet apart; however, use good judgment to determine best locations. Ceiling-hung traps are necessary for night-flying insects, such as those found in loading dock areas. Place the traps about 5 feet from the floor, attached to the wall, and at least 5 feet from open product so that contamination will not occur. These

traps must be checked at least weekly for the number and species of insects collected. Ultraviolet (black light) non-electrocuting traps are also useful for collecting many stored products pests.

d. Pheromone traps. Pheromone traps have several advantages. They can be placed inside equipment and areas that shelter pests, can compete with food odors, do not require additional expense to operate, work 24 hours a day, will detect low populations (often before management knows there is a problem), and are easier to count, because traps are species specific. Trap placement and maintenance are essential in using pheromones successfully. Indoors, traps should be placed 30 feet away from doors and in corners or on support posts in areas when insects have been noted, sanitation is poor, or there is a high potential for infestation (i.e., receiving areas). Protect the traps from dust, moisture, and damage. For crawling insects, place the traps from floor level to 6 feet off the floor. Use a grid pattern 25-50 feet apart to start, remove traps not catching anything, and concentrate traps in areas of insect activity. For monitoring, use 1 trap per 30,000 to 100,000 cubic feet of space. For mass trapping, use 1 trap per 3,000 to 10,000 cubic feet.

Outside, pheromone traps can be used to catch insects before they enter a facility. Place traps about 50 feet apart and use only at temperatures above 65^0F, since pheromone activity is temperature dependent. Traps must be protected from the elements (rain, wind, direct sunlight).

e. Open-package inspection. If insects or other subsistence pest problems are discovered or suspected, an open-package inspection must be performed; to ascertain if an infestation or contamination does exist; to determine the number of insects per pound inspected; and to obtain samples for veterinary identification. These inspections should always be conducted in the presence of veterinary personnel and the supervisor of the subsistence storage facility. This type of inspection is usually conducted subsequent to an infestation/contamination being detected. The samples should be taken from areas most likely to be infested/contaminated. The most likely areas would be the outermost containers on the top and bottom of stacks or the areas closet to an established problem area. If the product being inspected has a history of frequent infestation/contamination, or has been stored near infested/contaminated products, additional samples should be inspected. Use the entire contents of an individual package for open-package inspections for units of 10 lbs or less. Use a 3 lb sample for open-package inspections for units of greater than 10 lbs.

Samples are screened for insects on the examination table in one of two different methods: (1) by thinly spreading out the sample on the examination table covered with light colored disposable paper, or by (2) shaking the product through the proper sieve (100 mesh works well), or in the case of pasta or certain cereal products, shaking the insects through the larger hole size sieve.

A high intensity light source should be used for both methods. Isolate all insects found using forceps or a brush dipped in alcohol.

5. Surveillance data and action threshold.

All insect infestations must be identified, controlled, and reported to enable DoD to accurately assess and manage stored subsistence losses. Inspectors will submit a typed or printed DD Form 1222, "*Request for and Results of Tests*", and insect specimens to personnel providing identification services.

Military Standard 904A (MIL-STD-904A), 1984, "*Guidelines for Detection, Evaluation, and Prevention of Pest Infestation of Subsistence*" provides specific action thresholds for stored products (=subsistence) insect infestation and contamination. These guidelines delineate strict procedures to be used by veterinary and preventive medicine personnel whenever a pest infestation or contamination is detected upon receipt of subsistence from a commercial source or is detected during storage. **Specific guidance is as follows for insect infestation found within the product itself:**

a. When an infestation is found to include living or dead larval stages of an insect species belonging to the genus *Trogoderma* or other dermestids, 1 insect within the product itself (not external) shall be justification for the condemnation of the lot.

b. When an infestation if found involving living or dead insect species belonging to the genus *Tribolium*, an average of 3 insects or more per pound within the packages inspected shall be justification for the condemnation of that lot.

c. When an infestation is found to involve insects other than those belonging to the genus *Trogoderma* (or other dermestids) or *Tribolium*, an average of 7 living or dead insects per pound of product, in the lot being inspected, shall be justification for the condemnation of that lot.

6. Preservation.

Immature beetles (larvae) should be killed by placing them in boiling water for 1-5 minutes, depending on the size of the specimens. They can then be preserved in 80% ethanol. Adult beetles may be killed and preserved in alcohol, but it is recommended that they be killed in alcohol or in a killing jar and then mounted on pins. Specimens smaller than 5 mm should be glued to points, using care not to conceal ventral characters. Very tiny beetles can even be mounted on microscope slides.

Kill immature moths (larvae) by immersing them for 1 minute in hot water (nearly boiling) and preserve them in 80% ethanol. Adult moths should be killed in a

killing jar taking care to avoid rubbing off the wing scales. The adults can then be placed in individually in a separate, labeled envelope. For permanent collections, adult moths should be pinned with the wings spread in the standard manner so that all important features of wind pattern and structure may be seen.

CHAPTER 17: Hard Ticks (Family Ixodidae)

1. Military Importance.

Ticks are among the most important of all arthropod vectors of disease worldwide. They are second only to mosquitoes in the number of diseases they transmit to humans. Ticks generally affect military operations in two ways: (1) directly, by tick bite, tick paralysis and the accompanying psychological stress; and (2) indirectly, by tick-borne disease transmission. Various tick-borne diseases such as Lyme borreliosis, Rocky Mountain spotted fever, boutonneuse fever, tick-borne relapsing fever, tick-borne encephalitis, Siberian tick typhus, babesiosis, and others have afflicted military personnel throughout history. In January 1992, 169 U.S. soldiers participated in a 10-day training exercise with the Botswana Defense Force near Shoshong, Botswana. Within two weeks of their return to home station, approximately 30% of the deployed soldiers sought medical attention with symptoms of fever, headache, and regional lymphadenitis. A retrospective epidemiological study concluded that the disease responsible for these symptoms was tick-borne boutonneuse fever, of which the causative agent is *Rickettsia conorii*. The attack rate of 23% (39 of 169 soldiers) confirmed the occurrence of a large focal outbreak of a spotted fever group rickettsiosis among American soldiers participating in a short field training exercise in Botswana. In addition to adversely affecting military personnel tick-borne diseases have also impacted military working dogs. A tick-borne epizootic of tropical canine pancytopenia affected military working dogs in Southeast Asia during the 1960s, with at least 160 military working dogs killed by the disease.

2. Background.

Ticks are capable of transmitting a greater variety of infectious agents to humans and domesticated animals than any other group of blood-feeding arthropods. Disease organisms transmitted by ticks include viruses, spirochetes, rickettsia, anaplasmas, bacteria, piroplasms and filariae. Tick density does not necessarily coincide with disease transmission. In other words, disease transmission can occur in an area with relatively few ticks present or little or no disease transmission can occur in an area with millions of ticks present. However, when both conditions occur simultaneously (e.g., numerous ticks and high probably of disease transmission), military operations can be severely limited by incapacitating disease amongst personnel as well as annoyance from tick bites. Still another problem associated with feeding ticks is dermatosis and related reactions such as inflammation, itching, swelling and ulcerations at the site of the bite.

3. Geographic distribution and habitats.

Blood sucking ticks are found in virtually all terrestrial regions of the earth. Most species, however, flourish in a single optimum habitat type (e.g., deciduous forest, savannah, dry upland forest). The presence of suitable habitat, hosts, adequate rainfall during the period of seasonal activity, and winter temperatures above the species survival threshold are the dominant factors affecting the tick's zoogeographic range.

Hard ticks are commonly classified as being "one-host," "two-host," "three-host," or "many-host" ticks. One-host ticks, which are not common among ticks, complete all feeding and molting on a single animal. Two-host ticks complete the molt from larva to nymph on the host, then the engorged nymph drops to the ground following feeding, where it molts to the adult stage. The adult must then find the second host. Three-host ticks leave their host after engorging at each life stage. All molts take place off the animal. Most hard ticks are of the three-host type. Many-host ticks feed on a number of different animals during their life cycle, with the adult feeding several times. This behavior is typical of most of the soft ticks, discussed in the next chapter.

Tick dispersal by crawling is generally negligible. Adult tick dispersion is believed to be an insignificant consideration in determining population densities over time. Several studies have shown that adult ticks disperse a total distance of less than 5 meters. However, the overall rate of host acquisition by ticks is variable (e.g., <10% for *Dermacentor variabilis* larvae compared to up to 50% for *Ixodes scapularis* nymphs and adults), indicating the loss of ticks from sites in this manner may be significant. This information indicates that some tick populations are effectively closed, while others may be open.

The density of questing ticks infected with human disease agents frequently have been used to estimate spatial and temporal disease (e.g., Lyme disease, tick-borne encephalitis) risk. Although it is clear that ticks exhibit seasonal patterns of activity, it is unclear how highly conserved they are from year to year. Long-term population studies are generally lacking. Efforts to relate tick abundance to abiotic (e.g., weather, rainfall) and biotic (e.g., host density) factors have not been successful to date. However, it has been shown that when rainfall is less than half the historical average at a site, tick populations are significantly depressed. Natural fluctuations in tick populations should directly influence human risk of infection with tick-borne diseases. Tick life-spans are usually measured in years rather than days or weeks. Specific life stages, adults in some species, larvae and nymphs in others, readily survive from one year to the next.

4. Procedures.

The Armed Forces Pest Management Board Technical Information Memorandum 26, "*Tick-Borne Diseases: Vector Surveillance and Control*", is an excellent document for information on specific surveillance procedures that is available for download from the APFMB web site at URL **http://www.acq.osd.mil/afpmb/**

a. Walking survey.

The "walking survey", which consists of collecting ticks seen directly on oneself after moving through the vegetation provides the best information on the threat to humans from ticks. However, there are many biases inherent in this method. The major biases include differences in individual attractiveness and variability in the extent of contact with the vegetation. This technique can be used for adult *Dermacentor, Ixodes, and Amblyomma* ticks, which cling to the tips of leaves on low vegetation, usually on the underside of the leaves so only the legs can be seen from above the vegetation.

White, 100% cotton clothing (pants and shirt, or coveralls, and socks) should be worn to highlight ticks encountered. Surgical stocking net (NSN 6510-00-559-3159) can also be used; it is pulled over the legs of the regular battle dress uniform (BDU). It can then be taped down around the lower part of the boots to prevent ticks from getting under the material. This method is advantageous because there is no special clothing requirement.

b. Dragging or Flagging survey.

The cloth lure method involves using the "flag" technique (moved laterally by the investigator) or the "drag" technique (pulled behind). These techniques are effective in collection of larvae, nymphs, and adults of many three-host ixodid ticks. However, they are seldom used for one-host ticks since the larva is the only stage that is active on the ground and this is susceptible to detection by such off-the-host collection methods. Nymphs and adults of one-host ticks spend their entire parasitic periods attached to a host. Tick dragging or flagging collects representative samples of the hard ticks present indicative of the actual exposure a person active in the area might experience. This technique can be very manpower intensive and may yield few ticks in areas of low tick densities. However, it is the most practical method to quickly survey or "spot check" an area.

The ticks, which respond to mechanical stimuli, attach themselves to the cloth during the sweep. The cloth lure technique consists of counting the number of ticks caught by unit time (hours) or by surface area covered (hectares) and is generally done by extrapolation of results obtained from a period shorter than 1 hour or a surface area smaller than 1 hectare. However, the resulting tick

density data are generally not comparable between sites because sampling parameters (e.g., vegetation type) differ widely.

The cloth drag technique consists of a cloth surface area fixed at 1 m^2 (1 by 1 m). This size allows unrestricted passage across all types of vegetation and facilitates density calculations. A variety of white cloth types with a nap that facilitate tick attachment, have been used: cotton, muslin, woolen flannel, "molleton" (soft thick cotton), and toweling (a towel, sponge cloth). A 1.3 m dowel or strip of wood is attached to the leading edge of the cloth so that the entire cloth surface is in contact with the vegetation. A weighted rod (e.g., steel rod) is sewn into the trailing edge for added weight to ensure that the cloth makes contact with the underlying vegetation. The cloth drag is then pulled behind the surveyor with a 2-m rope handle attached to each end of the dowel along the leading edge. The person conducting the tick drag must walk at a slow pace (do not exceed a pace of 50 cm or 20 inches per second). This slow speed of passage has been shown to be optimal for tick collection. Each sample is conducted along a vegetation strip of 100 m. A path length of 100 m is generally used because it allows for empirical sampling equally useful during periods of high and low tick abundance. Cloth or clothing should be examined for ticks every 10 m. During high tick abundance or dense vegetation, this procedure limits the detachment of ticks (because they are physically removed by rubbing against the vegetation or dropping off after having recognized that the lure is not the host); during low tick abundance, this length (10 m) provides a sufficient number of ticks. However, previous studies on *I. ricinus* nymphs and *I. pacificus* adults found that ticks are lost from the tick-drag while it moves through a sampling transect , and specific correction factors (e.g., based on a drop-off rate of 0.0607/m dragged for *I. pacificus* adults) can account for this loss. This drop-off rate may be applicable if transects longer than 10 m are used. After each sub-sample, the ticks are removed from the cloth, placed into collection vials, and subsequently counted and identified.

The cloth drag can also be held nearly vertical (with respect to the ground surface) to simulate persons brushing against vegetation as they walk. This method is also known as "flagging". This method is best used to sample vegetation along sides of trails and campsites.

Anyone pulling a tick drag or using a tick flag is in effect engaged in a "tick walk". Ticks attaching to a person conducting any form of surveillance should not be included in the sampling estimate. (However, when using the drag method, the collector is actually the first to encounter questing ticks and therefore reduces the likelihood that specimens will attach to the drag).

Flag- or drag-sampling overall efficiency is estimated at 5%. Previous estimates include 5.9% for *I. pacificus*, 7% for *Dermacentor occidentalis*, 8-20% for *D. variabilis*. This indicates a 20-fold underestimate of actual population size.

Thus, the mean relative densities of ticks must be multiplied by 20 to obtain realistic population estimates for the sampling site.

Many tick species do not occur randomly, but rather show clumped distributions. Highest tick densities are generally correlated with areas of high mammal activity, such as animal trails or bedding sites. Edge habitats, as where forests open to fields, trails or clearings, may have the highest tick concentrations. The influence of tick hosts and associated "micro-areas" inside the study area, such as favored animal resting sites and animal paths, must be considered. Therefore, sampling methods (e.g., transect or random) must be determined before sampling can proceed. Recent studies indicate that transect and random sampling methods do not produce statistically different tick numbers.

The sampling methodology must also consider the **minimum sampled surface area required** (sampling representativity), randomness and non-selectivity. Sampling should be designed based on the smallest possible surface area, that is the smallest number of samples per collection required to give a representative sample of the area. In relatively homogeneous (i.e., random spatial distribution) areas, five sub-samples are sufficient to obtain a mean number of ticks per 10 m^2. Tick density estimates should be converted to number of ticks per square meter to allow for comparison over time or with other areas. This methodology conforms to the three indispensable criteria for correct sampling: representativity, randomness, and non-selectivity. Such a methodology provides a valid estimate of relative tick density and so will enable the comparison of results collected in other sites.

Sampling time to survey is best during late morning (1100-1230) and during the afternoon (1500-1630). Time of day and weather conditions affect tick activity and the number of ticks collected. Early mornings may not be a good time for tick collection because of overnight dew and low temperatures. Dragging and tick walks should not be done in the rain, when vegetation is wet, or during times when the air temperature is less than 54^0F (13^0C). These conditions usually reduce tick activity and questing and the resultant effectiveness of these methods.

Pick off all ticks and place them into the collection vial. Remove adult and nymphal ticks from the cloth with a pair of tweezers/forceps. Alternatively, a 2-inch wide piece of tape (or an adhesive lint roller) is a good method for quickly removing large numbers of ticks. The tape or lint roller's adhesive sheet can then be marked with the date, time, location, collector's name, and drag distance. Adhering the tape to the inside of a sealable plastic bag (Ziploc®) containing a piece of moist (not dripping wet) paper towel will keep ticks alive for weeks if they are kept cool. The ticks can be counted and tentatively identified while still adhering to the adhesive surface, and they can then be removed for further processing.

c. Carbon Dioxide Trapping.

For many tick species, carbon dioxide (CO_2) trapping can yield the most ticks per man-hour expended. This technique relies on the ability of ticks to sense CO_2 and move towards the source. Carbon dioxide traps have proved to be effective for actively questing tick species such as *Amblyomma* spp. and American *Ixodes* spp. For those tick species that adopt the sit-and-wait strategy, such as *Ixodes ricinus* (Lyme disease and tick-borne encephalitis vector in Europe), a fixed trap is less useful, except when using multiple traps for extended periods (at least 7 days).

Dry ice is placed in vented, insulated containers. Tape can be attached, sticky side exposed, around the perimeter of the trap to capture attracted ticks. One-half pound of dry (0.25 kg) ice will last about 2 hours at 80^0F (27^0C) using an insulated container. For rapidly moving tick species, a piece of dry ice can simply be placed on an inverted pie pan on a white sheet. Setting more than one trap at each collecting site will provide a more reliable estimate of the actual population.

Record date, site (use grid coordinates if possible), time traps were set, time ticks removed, species and numbers of ticks present for each of the traps at each site. Note: If, upon returning to count ticks, you discover that all the dry ice is gone from the trap, disregard the data from that trap and use only the data from the remaining traps to calculate the trap index. Calculate a trap index (TI) for each site to adjust results for differences in time that traps were operating at various sites. The TI is calculated by adding the total counts from all traps at one site and dividing by the number of traps (usually 3) to get the average catch for the site. This number is then divided by the time (in hours) that the traps were operating. Time in hours is calculated by dividing the number of minutes of trap time by 60 minutes. For example: 3 traps were set up at 0800 hours and ticks were counted at 1000 hours. The tick counts recorded were 47, 13, and 30. Since a total of 90 ticks were trapped by the 3 traps in 2 hours, the resulting TI would be 15 ticks per trap hour [(90/3)/2]. **Recent studies have shown that when all stages of ticks are counted, the number of ticks found on a trap per hour approximates the number that would be expected to attach to a human who remained in that spot for one hour.**

d. Host trapping and examination. Host trapping is the most accurate assessment of a local tick population, when appropriate hosts are sampling. Difficulty in catching some wild hosts is the limiting factor with this surveillance technique. CAUTION: Since the handling of rodents places one at risk for hantavirus exposure, procedures in the CDC's "Methods For Trapping And Sampling Small Mammals For Virologic Testing" (AFPMB TIM 40), "Protection From Rodent-Borne Diseases With Special Emphasis On Occupational Exposure To Hantavirus" (AFPMB TIM 41), or similar protocols should be closely followed to minimize risk and standardize procedures.

e. Burrow swabbing. This method is only effective for collecting ticks that inhabit animal burrows (e.g. *Ornithodoros parkeri*). The swabbing device consists of a flexible metal cable (plumber's snake) or similar device that has a metal clamp attached to one end. A square piece (swab) of cotton diaper flannel (15-20 cm, 6-8 inches in width) is attached to the clamp and the swab and cable are inserted into each burrow. Once the cable is forced as far down the hole as possible, it is left for approximately 30 secs before being gently retrieved. After retrieval, the flannel swab is placed on the ground near the burrow entrance and inspected for ticks. Remove the ticks from the flannel and place in glass or plastic vials for transport.

5. Surveillance data and action threshold.

Most threshold levels have been based on the more accurate monitoring method involving the use of dry ice. A count of 0.65 ticks/hour of dry ice exposure is considered the action threshold level. Tick-bite complaints may also be used to trigger action.

6. Preservation.

Forcible removal of attached ticks often results in damaged or lost capitula (mouthparts), making identification even to genus difficult. Flat, unfed ticks collected by dragging or flagging are the easiest to identify. With engorgement, it becomes progressively more difficult to see the capitulum and scutum clearly. Ticks collected from animals are often encrusted with dried blood and exudates or have host tissue attached to their mouthparts. Live ticks with attached host tissue will clean themselves within an hour or two if kept in a tube. Ticks encrusted with blood or exudates can be cleaned with 70% ethanol with a soft artist's brush on which the bristles have been cut short to uniform length.

All life stages of hard ticks (larva, nymph and adult) are usually preserved in tubes or vials of 70% ethanol and accompanied inside by a permanent label recording details such as: exact locality, host, habitat, collector and date. Live ticks can be placed directly in preservative fluids without needing to be killed first in hot water. Completely fill the tubes or vials with the alcohol to eliminate all bubbles thus preventing specimen movement and damage.

For identification, adult ticks should be examined dry under a dissecting microscope, preserved specimens being allowed to dry so they are free of any surface sheen produced by the ethanol. Smaller larvae and nymphs are best mounted on microscope slides and examined under a compound microscope for confirming the species identification.

CHAPTER 18: Soft Ticks (Family Argasidae)

1. Military importance.

Soft ticks generally affect military operations in two ways: (1) directly, by tick bite and the accompanying psychological stress, and (2) indirectly, by disease transmission.

2. Background.

Soft ticks play an important role in the transmission of diseases to humans and animals. Diseases transmitted by soft ticks include: (1) African tick-borne relapsing fever, caused by *Borrelia duttoni* (e.g., *Ornithodoros moubata* in African mud-hut dwellings); (2) North American tick-borne relapsing fever caused by *B. hermsi*, contracted by humans visiting or sleeping in rodent-infested cabins; (3) and viral encephalitides transmitted by nest- or harborage-infesting *Ornithodoros* spp. infesting sea birds, shore birds or other roosting birds.

Adult female soft ticks feed and lay eggs several times during their lifetime. Soft ticks may also undergo more than one nymphal molt before reaching the adult stage. With few exceptions in the larval stage, soft ticks do not firmly attach to their hosts for several days like hard ticks.

Most soft ticks are “multi-host ticks.” Argasids feed briefly, usually at night, and they leave the host promptly; they are not often collected on the host. Feeding lasts from 15 minutes to 2 hours. Larvae feed once, nymphs and adults feed twice or more.

3. Geographic distribution and habitat.

In contrast to hard ticks, soft ticks are limited to their specialized habitats, resulting in a more focused distribution. Many soft tick species thrive in hot and dry climates. Argasids have an exceptional ability to resist dehydration and starvation. Almost all species live together with their host in the shelter site. Such habitats include caves, burrows, bird colonies, old farm buildings and churches. In most situations, a typical soft tick feeds on a single host species during its nesting and hibernating season. However, in places where hosts are abundant, such as chicken houses or pigeon roosts, feeding may occur at any time of the year.

4. Procedures.

a. Handpicking. Handpicking soft ticks in their natural habitat is a crude method of survey. However, this method is the only practical means to encounter some species. Many species hide within or very near the roosting

quarters of their natural hosts and feed for very brief periods while the host is inactive. For example, *Ornithodoros* ticks (the vector of relapsing fever in humans) are most readily detected by examining the nesting sites of their rodent hosts. A recommended procedure is to examine 10 such situations (e.g., rodent burrows) with the aid of a strong light. A flushing agent (e.g., d-Phenothrin, NSN 6840-01-412-4634) can be sprayed into cracks and crevices and other hiding places to assist in surveying for soft ticks.

b. Collecting nesting material. Nest-inhabiting species of soft ticks can be found by placing nest material of the host on a mesh in a large funnel (a Berlese Funnel). Essentially, this method involves placing a bright light over the funnel – the ticks move downwards away from the light and are collected in a container below.

c. Burrow swabbing. This method is effective for collecting soft ticks that inhabit animal burrows. The swabbing device consists of a flexible metal cable (plumber's snake) or similar device that has a metal clamp attached to one end. A square piece (swab) of cotton diaper flannel (15-20 cm, 6-8 inches in width) is attached to the clamp and the swab and cable are inserted into each burrow. Once the cable is forced as far down the hole as possible, it is left for approximately 30 secs before being gently retrieved. After retrieval, the flannel swab is placed on the ground near the burrow entrance and inspected for ticks. Remove the ticks from the flannel and place in glass or plastic vials for transport.

d. Dry ice attraction. Dry ice (CO_2) bait traps are useful in collecting soft ticks from animal burrows, particularly burrow-inhabiting species of the genus *Ornithodoros*. The trap is constructed from a cardboard mailing cylinder, 3-5 centimeters in diameter and 8-12 centimeters long, with a screen cone entryway through a screw-cap lid. The device is inserted 0.5-2.0 meters into an animal burrow. Attaching a tube to a dry ice reservoir at ground level provides the CO2 that attracts the ticks to the trap.

5. Surveillance data and action threshold.

In hand-picking, average counts of fewer than 3 ticks per location are considered light; 3-10, moderate; and over 10, heavy.

6. Preservation.

Collected soft ticks are usually preserved in tubes or vials of 70-80% ethanol and accompanied inside by a permanent label recording details such as: exact locality, host, habitat, collector and date. Completely fill the tubes or vials with the alcohol to eliminate all bubbles thus preventing specimen movement and damage. Immature ticks may need to be mounted on slides and examined under a compound microscope for confirming the species identification.

Chapter 19: Parasitic Mites

1. Military Importance.

The direct physical damage done to humans and animals by attacks of parasitic mites include itching, dermatitis with crusts of dehydrated serum or blood, enlarged lesions, loss of hair, damage to skin, and a predisposition to infection by bacterial and fungal disease agents. Trombiculid mites are an important vector of *Orientia* (formerly *Rickettsia*) *tsutsugamushi* causing scrub typhus, found in southeast Asia and nearby islands. In recent years, scrub typhus cases have been reported at much higher rates and thus poses a significant threat to non-immune military personnel.

2. Background.

There are thousands of species of mites; most of them are vegetable and detritus feeders or are predaceous on small invertebrates. Many others are parasitic on reptiles, birds, animals and humans, and some are important disease vectors.

There is increasing evidence that common allergy causing mites (e.g., *Dermatophagoides farinae, D. pteronyssius, Euroglyphus maynei* and *Blomia tropicalis*) are sources of potent allergens in house dust. These mites have very high reproductive potential and population growth rates. Scrub typhus is a focal disease that persists only in areas that have certain well defined features. The basic elements that must be present for a focus to persist are *O*. *tsutsugamushi*, *Leptotrombidium deliense*-group mites, certain small mammal hosts (especially field rats of the genus *Rattus*), and “scrub” or transitional vegetation . Also, dermanyssid mites are known to be vectors of at least two rickettsial diseases of humans: *Ornithonyssus bacoti* has been involved in the experimental transmission of typhus fever, and *Liponyssoides sanguineus* is a vector of *R. akari*, the etiological agent of rickettsial pox. Other members of the family Dermanyssidae are associated with St. Louis encephalitis in humans.

3. Geographic distribution and habitat.

Many mite species are found worldwide. Optimal mite habitats have high humidity, moderate to high temperatures, abundant food supplies or hosts, and moist soil conditions that provides abundant prey for the free-living nymphal and adults stages. Allergy-causing mites are most often found in high use areas where shed skin scales collect and serve as a food source, and the relative humidity is above the critical levels for sufficient time to satisfy their water requirements.

4. Procedures.

Methods of collecting mites vary with the individual species, but the following general methods are usually applicable.

a. Skin scraping. In the case of scabies or mange caused by mites, the usual procedure is to scrape the suspect area with a scalpel or pocket knife. If infestation with *Sarcoptes scabiei* is suspected, scrapings must be deep enough to expose the moist layers of the skin, since these mites burrow beneath the surface or penetrate the hair follicles. The entire scraping can be sent to a diagnostic laboratory in an ointment tin or vial or the mites can be removed from the scraping and either be preserved or mounted on slides for later microscopic identification of the species. The host and the appearance of the lesion may serve as a guide as to the mite involved and the kind of scraping required.

b. Handpicking and aspiration. Mites that are readily visible may be collected with an aspirator or a small camel-hair brush dipped in light oil or water. Such procedures are especially useful for those mites that spend considerable time off the host in cracks and crevices, as in the case with many fowl mites. Bedding, nests, and associated debris should also be examined.

c. Incision of nodules. Mites of the genus *Demodex* require special detection and collection techniques. These mites can be a pest of dogs and are usually found inside various-sized nodules located within the dermal layers. Dogs can be severely affected by *Demodex canis*. The disease usually takes a so-called “squamous” form characterized by red, hairless areas, especially around the eyes, elbows, hocks, and toes. Alopecia may spread to other parts of the body, and the skin becomes copper-colored or bluish. In some infestations, pustules or nodules occur, characterized by an offensive odor. The skin becomes thickened, wrinkled, and inflamed, accompanied by severe itching. The actual cause of the disease is a *Staphylococcus* infection spread by the mites. Death may occur in severe cases. Diagnosis is made by finding mites in the pustules or nodules. Mites are often present on healthy dogs, and manage develops only as a result of some predisposing condition. If the nodule contents are intended for subsequent bacteriological culture, the skin should be cleansed with alcohol, the nodules incised, and the cheesy contents expressed directly into sterile vials. If entire skin sections are to be submitted for laboratory examinations, these should also be sent in sterile, stoppered vials. Nodule contents or whole nodules should be refrigerated during storage or shipment.

d. Monitoring with black squares. Larval trombiculids (known as chiggers) can be monitored using 12-inch squares of black paper or plastic that are placed on the ground in suspected chigger habitat for 1-5 minutes, after which the total number of chigger mites that congregate on the black squares are counted. Locate plates about 100 meters apart. If nothing else is available, the black surface over the toes of combat boots can be used to visualize

crawling chiggers. Another efficient method of sampling chiggers is to trap their rodent hosts and examine them for the presence of the mites. The chiggers are usually yellowish or orange and concentrated in the ears or in the groin area of the rodent.

e. Berlese funnel. A Berlese funnel can be used to separate mites from suspected living media (dust and debris). The mites migrate downward as the surface dries and are collected in a jar of 70% ethanol beneath the funnel. Although there is some mortality due to mites dying while crawling down the sides of the funnel, large numbers can be collected.

5. Surveillance data and action threshold.

Decisions to control chiggers in nuisance situations are arbitrary, based on complaints or logistics. Generally, any action taken is aimed at personal protection rather than chigger control.

6. Preservation.

Mites collected for identification and culture should be delivered to laboratories free of tissue debris and in large numbers. To cause mites to depart from the tissue debris, place the suspected skin scraping in a deep beaker and then set the beaker inside an open petri dish containing enough water to cover the bottom. The beaker and dish should then be placed inside an incubator at approximately 35^0C and 80-99% relative humidity. The mites will leave the tissue debris within a few hours and they can be easily picked off with a moistened probe or fine brush. Also, mites may be trapped in the water moat. There they can be picked up, sometimes in clumps or aggregate of considerable size. They then can be transferred into a vial for preservation.

Unopened nodules may be preserved and shipped in 70% ethanol or 10% formalin for histological sectioning and mounting. They may also be quick frozen and shipped in containers of dry ice. Under no circumstances should fresh sections of skin containing mites of any species be mailed or shipped without preservation or refrigeration. The tissue fragments would be certain to undergo bacterial and fungal decomposition in a matter of a few days, which would render further examination impossible.

If mites are desired alive for culturing or other purposes, they may be collected on dry hair or wool or they may be isolated. Then they are shipped or mailed in suitable containers in which moisture is not excessive. If proper conditions (comparatively low humidity and normal room temperature or below), mites may remain alive and active in the mail for several days. If humidity is high, particularly in sealed containers, excessive fungal growth can be expected.

CHAPTER 20: Venomous Arthropods

1. Military Importance.

Stings, bites or other contact with arthropods can result in dermatitis, severe neurological or cytological symptoms, localized or systemic allergic reactions and secondary bacterial infections. Troops should be taught to recognize local venomous arthropods and ways to avoid them when living in the field.

2. Background.

Females in the Order Hymenoptera are the only insects that possess a true stinger. Stinging insects include yellow jackets (*Vespula*), Old World hornets (*Vespa*), wasps (*Polistes*), honey bees (*Apis*), bumble bees (*Bombus*), and fire ants (*Solenopsis*).

Reactions to stings and bites and stings fall along a continuum ranging from virtually no reaction to localized reactions to systemic involvement. Hypersensitivity to arthropod venom ranges from mild, localized cutaneous reactions to severe systemic anaphylaxis and death. In addition to anaphylaxis, death can result from toxic reactions to multiple insect stings. Death due to toxicity of insect venom occurs most frequently in young children and the elderly.

Africanized honey bees (AHBs) deserve special mention as they are extremely sensitive to the slightest disturbance and the hive responds with massive and persistent stinging attacks. Although the AHB does not attack unprovoked, it is very defensive of its colony. AHBs are the same species of the common honey bee (*Apis mellifera*), but are a hybrid resulting from crossing a tropical (Africa) strain and a temperate (European) strain of honey bees. They are difficult to manage and have a strong tendency to leave existing hives (abscond) and settle elsewhere. AHBs are a real and significant threat for those who must live with them. However, they can be satisfactorily dealt with as long as the appropriate precautions and control measures are taken (see Technical Information Memorandum 34. *Bee Resource Manual with emphasis on The Africanized Honey Bee*, 1995. (Current version is available from the Armed Forces Pest Management Board. and is downloadable guidance at URL **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5” x 11” format).

The most toxic arthropod bites are associated with spiders and scorpions. In terms of human death and sickness resulting from bites, the most dangerous spiders are found in 4 genera: *Atrax* (Dipluridae), *Harpactirella* (Barychelidae), *Loxosceles* (Sicariidae) and *Latrodectus* (Theridiidae). *Atrax robustus* is the Sydney funnel-web spider of Australia. The genus *Harpactirella* of South Africa contains 11 venomous species. *Loxosceles reclusa* is the brown recluse spider and *Latrodectus mactans* is the black widow spider. Tarantulas

(Theraphosidae), although intimidating in appearance, are infrequently sources of envenomization. In addition, spiders can also cause allergic reactions through the inhalation of body hairs or scales.

Centipedes may grow to impressive lengths (over 8 inches) but are rarely a serious threat to human health. The front pair of legs is adapted to form fangs attached to venom glands, which the centipede uses to inject poison into its prey. A few of the larger species, such as *Scolopendra morsitans*, may inflict an unpleasant bite to humans. The bite can cause local inflammation; redness and swelling usually disappears in 4-5 hours. However, human death has never been reported from a centipede bite.

Fewer than 25 species of scorpions, all in the family Buthidae, posses a venom that is life threatening to humans. Scorpions inject venom with a stinger on the tip of their abdomen, and some species can inflict a painful pinch with their pedipalps. They feed at night on insects, spiders, and other arthropods. During the daytime, scorpions hide beneath stones, logs or bark, loose earth or among manmade objects. In dwellings, scorpions frequently rest in shoes or clothing.

3. Geographic distribution and habitat.

Venomous arthropods are found worldwide. They occupy a wide variety of habitats. Crawling arthropods are more common in secluded places, such as in unused building or sheds and under rocks or wood. Flying, stinging insects (i.e., bees, hornets or wasps) can be found anywhere both, indoors and outdoors.

4. Procedures.

a. Visual inspection. A thorough inspection inside and outside the building is needed to determine the spiders involved in any particular situation, how they are entering, and any contributing conditions. Inspect outside, garages, crawlspaces, attics and basements looking for the presence of webs and moving items to check for spiders hiding underneath. The presence of web-building spiders is easier to detect than that of hunting spiders.

b. Sticky traps. Sticky traps are especially useful for determining activity involving active hunter species (e.g., wolf spiders).

c. Vacuuming. Vacuuming can be used to survey for and to physically control spiders and scorpions. Vacuuming is very efficient and effective, particularly when used in conjunction with a flushing agent to move arthropods (and webbing and egg sacs) from deep harborage. The vacuum must: 1) be small enough to be maneuvered easily (e.g., backpack device); (2) be able to withstand accidental suction of water, which is a distinct possibility in large kitchens; and (3) have a High Efficiency Particulate Air (HEPA) filter. Without a HEPA filter, pathogens, exoskeletons, feces, and other allergens will be

removed from protected sites and become airborne, causing potential health problems for residents and workers.

5. Surveillance data and action threshold.

There is little, if any, surveillance data available with which to judge infestation levels. Action may be taken against a spider infestation when it is considered a nuisance or health threat. The number of spiders or spider webs per unit area required before action can occur is arbitrary. Certainly, the confirmed presence of even one brown recluse or black widow spider is usually enough for most workers to take action. Contact with most venomous arthropods is rare occurrences, unless there is a nest nearby a human occupancy or workplace. Arthropod nests (i.e., wasp nest, bees nest, stinging ant nest) that are a local problem should be eliminated.

6. Preservation.

Accurate identification of spiders are especially important in the case of a reported spider bite. This will enable the treating medical staff to make appropriate treatment decisions based upon the knowledge of the particular spider species.

CHAPTER 21: Rodents

1. Military Importance.

Wild and commensal rodents are important carriers of human diseases that can quickly spread to humans and greatly affect military operations. Rodent-borne hantaviral disease is an emerging medical threat to military forces operating in many areas of the world. A hantavirus carried by the striped field mouse (*Apodemus agrarius*) caused about 3,000 cases of Korean hemorrhagic fever among United Nations troops during the Korean conflict. Over 20 acute Puumala virus infections were documented in US Army personnel during a 1990 field exercise in southern Germany. Several outbreaks of hantaviral disease occurred in 1995 as a result of the civil war in the states of the former Yugoslavia. Over 300 patients, most of them soldiers exposed in the field, were hospitalized in the Tuzla region (northeast Bosnia) with acute hantaviral disease. Hantaviral infections also occurred during the fighting in Kosovo. In 1999, hantavirus-infected rodents were collected from two US base camps in Kosovo. However, no infections or disease have been diagnosed in US troops who have served in Kosovo. In 1994, Sin Nombre virus (SNV), and the associated human disease, called hantavirus pulmonary syndrome (HPS) was identified in the southwestern United States. The rapid progression of the disease, its occurrence in previously healthy young adults, and the high case fatality ratio (initially about 70%) caused considerable alarm. Given the high case-fatality ratio among patients developing HPS (currently about 40%), this disease is a significant risk to military personnel training or operating in this area.

Leptospirosis has had a long association with US troops training and operating in endemic countries, especially Panama and Southeast Asia. A high incidence of leptospirosis antibodies (11%) was found in military recruits from northern Pakistan undergoing training near Mardan in 1984.

2. Background.

The diverse diseases caused hantaviruses in Europe and Asia are now grouped under the disease syndrome called hemorrhagic fever with renal syndrome (HFRS). Manifestations of HFRS vary from mild renal dysfunction to complete renal shutdown accompanied by capillary leak syndrome and hemorrhagic manifestations. Rodents are carriers of hantaviral diseases. HFRS is transmitted by both commensal and wild rodents (see Appendix 2 for details). Seoul virus, which causes mild-moderate HFRS in China and Korea, is spread by its principal host, the Norway rate (*Rattus norvegicus*). Hantaan virus causes severe HFRS in Asia and Russia. Dobrava/Belgrade virus, causes severe HFRS in the Balkans. Puumala virus found in Scandinavia, Europe, Russia, and the Balkans causes a mild HFRS.

The primary method of human infection with hantaviruses is believed to be inhalation of aerosolized virus, which is shed in urine, feces, and saliva of infected rodents. Exposure of infected rodents in closed, confined spaces may be particularly hazardous. Several cases of HPS in North America have apparently been the result of working or living in an indoor area with rodents. Human infection can also occur when virus or virus-contaminated materials are introduced into broken skin, conjunctivae, or mucous membranes, or perhaps when accidentally ingested with food or water. Infection may also be directly transmitted by bite. Personnel collecting blood or tissue samples from live or freshly-killed rodents are also exposed to virus in the blood or organs of infected animals.

The three important commensal rodents are the Norway rat (*Rattus norvegicus*), the roof rat (*Rattus rattus*) and the house mouse (*Mus musculus*). These rodents are responsible for the spread of many diseases through their bites and by contamination of human food with urine and feces. Commensal rodents cause significant economic damage by consuming and contaminating food and damaging structures and stored material by their gnawing.

Leptospirosis is caused by the spirochete bacterium *Leptospira interrogans.* It is an occupational hazard to rice and sugarcane-field workers, farmers, sewer workers, miners, veterinarians, dairyman, fish workers, and military troops. Outbreaks occur to those exposed to fresh water, stream, canal, and lake water contaminated by urine and tissues of infected domestic and wild animals. It is contracted by direct contact with urine of infected animals and occasionally by inhalation of droplet aerosols of contaminated liquids.

The bacillus *Streptobacillus moniliformis* causes rat-bite fever. Infection is transmitted by urine or secretions of the mouth, nose or conjunctival sac of an infected animal, most frequently introduced by biting.

3. Geographic distribution and habitat.

Commensal rodents occur worldwide and are commonly found in or near human habitations. Hantaviral diseases occur in many areas throughout the world. Seoul virus, which causes mild-moderate HFRS primarily in China and Korea, also occurs sporadically in many port cities around the world. Hantaan virus causes severe HFRS in Asia and Russia. Dobrava/Belgrade virus, which causes severe HFRS in the Balkans is a constant threat to military forces deployed in this region. Puumala virus found in Scandinavia, Europe, Russia, and the Balkans causes a mild HFRS.

Leptospirosis occurs worldwide in urban and rural, developed and developing areas, except polar regions. This disease is regarded as focally enzootic throughout South Central Asia, Central Europe, and Central and South America.

Rat-bite fever occurs worldwide, but is uncommon in North and South America and most European countries.

4. Procedures.

a. Commensal rodent visual surveys.

Commensal rodents may be found almost everywhere humans live or work. Rodents are nocturnal and secretive animals and are rarely seen during the day except when infestations are heavy. However, commensal rodents leave a variety of signs in the areas they frequent. Survey personnel should be on the lookout for the following signs that indicate their presence. Note: A flashlight is essential for conducting rodent surveys since signs of rodents are most often found in poorly lighted locations.

(1) *Sounds*. Sounds of gnawing, scratching, squeaking, and running in walls and above ceilings can be heard in buildings infested by rodents.

(2) *Droppings*. Droppings (feces) are found in places rats and mice frequent. Mouse droppings are rod shaped and 1/8 to ¼-inch long. Norway rat droppings are capsule shaped, ¼ to ¾-inch long and 1/16 to ¼-inch in diameter. Roof rat droppings are spindle shaped and up to ½-inch long.

(3) *Urine*. Rodent urine is not visible on all materials under natural light. Under ultraviolet light (black light) it fluoresces bluish white to yellowish white. Note: Lubricating oils and bleaches found in many detergents also fluoresce.

(4) *Rub marks*. Rodents accumulate dirt and oil deposits on their fur. As they travel, they leave smudges where they rub against pipes, beams, and openings. Rat smudges are much more conspicuous than those left by mice.

(5) *Runs and trails*. Runs occur in sheltered areas where rodents feel secure as they move. They appear as dust-free pathways within building or beaten paths outdoors. Like smudge marks, rat trails are much more conspicuous than those made by mice.

(6) *Tracks*. Footprints and tail marks may be found in dust and mud. Tracking patches made with flour or talc can be used to determine rodent presence in buildings. Dry soil dust can be used outdoors if protected from weather and disturbance.

(7) *Odor*. Mice produce a musky odor which an experienced person can differentiate from rat odor. Odor is probably not detectable when the rodent population is low and ventilation is good.

(8) *Gnawing*. Rodent gnawing results in small piles of wood chips around doors, baseboards, and windows; damage to stored goods and food product containers; and enlarged openings where pipes and wires penetrate walls. Freshly gnawed areas are lighter in color than un-gnawed material, and tooth marks may be apparent.

(9) *Burrows*. Holes and enlarged openings in walls are often burrow entrances. Norway rat burrows may be found around shrubbery and sidewalks, under foundations, and along stream banks.

(10) *Nests and food caches*. Mouse and rat nests, usually in the form of a loose ball of shredded cloth, insulation, paper or dry vegetation, may be found during cleaning of garages, attics, warehouses, and other storage areas. Nests are often built in furniture, inside large electrical appliances, and in vehicles that have been parked from an extended period of time. Rat and mouse food caches may be found during clean-up operations.

b. Rodent trapping.

CAUTION: The Centers for Disease Control and Prevention warns that the most important prophylactic measure for personnel who are trapping, handling, bleeding, or dissecting rodents is to be aware of potential routes of infection and carefully avoid conditions, which may lead to transmission. This information is available from AFPMB TIM 41, *Protection From Rodent-Borne Diseases with Special Emphasis on Occupational Exposure to Hantaviruses*, 1999, and is downloadable guidance available at URL **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5” x 11” format). Specific and detailed instructions for rodent trapping, especially for virologic testing, are available from AFPMB TIM 40, *Methods for Trapping and Sampling Small Mammals for Virologic Testing (=CDC Manual)*, 1995, and is downloadable guidance available at URL **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5” x 11” format). Small-mammal “removal” (not returned to site of capture) trapping procedures are briefly summarized below.’

(1) *Bait*. Rolled oats mixed with a small amount of peanut butter makes a good bait.

(2) *Traps*. Snap traps (mouse trap, NSN: 3740-00-252-3384 or rat trap, NSN: 3740-00-260-1398) or rodent glue boards (NSN: 3740-01-240-6170) can be used if rodents will not be processed for ectoparasite or hantavirus detection. If animals are needed to be collected alive, collapsible, self-closing (NSN: 3740-00-472-2743) traps, 12” x 6” x 6”, may be used for smaller mammals or large Sherman folding traps (NSN: 3740-00-431-1186) may be used for larger mammals.

(3) *Trap placement*. Traps should be set out before dark. Place trap lines in areas that are out of sight of roads, sidewalks, paths, or other areas of human activity. When possible, place traps near brush piles, fallen logs, burrows, abandoned car bodies, or other items that provide shelter. Indicate the beginning and end of each trap line with a small piece of surveyor's flagging marked with the trap line number. Place the traps in lines of 10-20 traps at approximately 5-meter intervals. Avoid placing traps in areas that will be exposed to direct sun.

(4) *Collecting captured rodents*. Traps should be checked as early in the morning as possible, especially in hot weather. Survey team members MUST wear protective clothing, including long pants and long-sleeved shirt, socks, boots, and heavy rubber gloves (refer to TIM 41 are mentioned above). Check each trap for evidence of capture or visitation. If the trap is sprung and contains a rodent (target species), mark the top of the cage and carefully place the trap into a plastic bag and tie it closed. If the trap contains a non-target species (e.g., toad, bird, etc.), carefully release the animal at the site of capture. Upon completion of the trap line, return the captured animals to the vehicle and complete the trap tally form. Do not reopen to the plastic bags once they are tied closed. Bags may only be opened after technicians have donned complete protective equipment at the processing site. Place the plastic bags containing captured rodents in the back of the field vehicle and transport them directly to the processing site. After placing animals in the vehicle, wash rubber gloves thoroughly in soap and water, then remove gloves and wash bare hands in soap and water.

(5) *Collecting ectoparasites and blood and tissues from captured mammals*. These procedures required MANDATORY precautionary measures and specialized skills that are outlined in detail in TIMs 40 and 41 (mentioned above). If blood and tissues are to be collected for virus isolation and identification, the reference laboratory in which samples are to be sent should be contacted for specific collection, handling, and shipping instructions.

5. Surveillance data and action threshold.

Rodent surveillance data can provide valuable information about rodent infestations such as: 1) presence or absence of rodents; 2) species present; 3) changes in populations over time; and 4) potential disease threat.

6. Preservation.

For positive identification of many rodent species, it is important to have specific identifications confirmed by a museum. Arrangements should be made well in advance for a reference laboratory or museum to accept, identify, and archive specimens. Variations may be made in the following procedure, but the 7-day fixing period should be strictly observed for safety reasons.

a. Leave carcasses in 10% formalin for 7 days before handling.

b. Rinse the carcasses for a few hours or overnight in running water and then place in a container of 70% ethanol.

c. Remove the carcasses from the or alcohol and wrap individually in moist cheesecloth or paper towels.

d. Place the individually wrapped carcasses in double zip-lock bags.

e. Place the zip-lock bags into larger plastic bags and pack them in a sturdy box for shipping.

f. Seal the box with fiber tape and send the quickest way possible.

g. Notify the laboratory of the shipment and follow-up to make sure it is received.

CHAPTER 22: Poisonous Snakes

1. Military Importance.

Military personnel are often stationed in or visit many parts of the world where poison snakebites can be a public health threat. The risk of being bitten increases during amphibious operations, especially in tropical and sub-tropical regions. During such operations the natural habitat of venomous snakes may be disturbed so that exposure to them is markedly increased. US military forces have never experienced disease non-battle injury rates from snake venom poisoning sufficiently high to jeopardize the outcome of an operation. Even during World War II, admissions for medical treatment due to such accidents were surprisingly few in number. This was true even in Burma, where snakebite was common among the civilian population. This situation is illustrated by data accumulated during 1944 and 1945, with overseas admissions due to snake and arthropod bites being 0.11/1,000 and 0.04/1,000, respectively. These figures of course gave small consolation to those who suffered such bites. The threat of snakebite may create a morale problem sufficient to delay an operation or cause unnecessary fear during its execution. Snakebites are uncommon in military forces and subsequent fatalities are extremely rare. However, snake venom poisoning does constitute a true medical emergency requiring immediate attention and considerable judgment in clinical management. For this reason alone, it is essential to possess accurate information concerning venomous snakes so that accidents can be avoided when possible, and effective treatment provided if necessary.

2. Background.

There are five families of poisonous snakes: Family Colubridae (colubrid snakes), Family Elapidae (cobras, mambas, kraits, and coral snakes), Family Hydrophidae (sea snakes); Family Crotalidae (pit vipers), and Family Viperidae (typical or Old World vipers).

In most parts of the world, bites by non-venomous snakes occur far more frequently than bites by venomous snakes. Since the differentiation is often difficult, all victims of snakebite should seek immediate medical attention. A difference of 30 minutes to 1 hour in initiating treatment in some snake venom poisonings (especially with elapids) may make the difference between life and death. Whenever possible, the offending snake should be killed and brought with the victim to the medical treatment facility for identification. In most cases, there is great clinical value in differentiating between bites of venomous and non-venomous snake species.

Bites by the vipers (Old World vipers, pit vipers of Asia, eastern Europe, and the rattlesnakes and related species of the Americas) usually result in one or two relatively large puncture wounds of varying depth, depending on the size of the

snake, the force of its strike and the location of the bite on the victim's body. Bites by the elapid snakes (cobras, mambas, tiger snake, taipan, coral snakes, and related species) usually produce one or two small puncture wounds, although occasionally there may be one or two additional punctures. Sea snake bites are characterized by multiple (2-20) pinhead-sized puncture wounds. In some cases the teeth may be broken off and remain in the wound.

It is important to understand that a person can be bitten by a venomous snake and not be poisoned. In 3-40% of the bites inflicted by venomous snakes, no signs or symptoms of poisoning develop. This important fact should always be considered before specific treatment is started. However, any snakebite associated with immediate (and sometimes intense) pain, and followed within several minutes by the appearance of swelling and subsequent edema is usually diagnostic of snake venom poisoning by a viper. Elapid envenomization is followed by pain of minor intensity that appears 10-30 minutes after the bite and swelling (appearing 2-3 hours after the bite) is usually limited to the area of the wound.

3. Geographic distribution and habitat.

North American poisonous snake fauna includes 2 species of coral snakes, the closely related copperhead and cottonmouth, and 15 species of rattlesnakes. South America is home to poisonous snakes from five genera (*Micrurus, Letomicurus, Crotalus, Lachesis,* and *Bothrops*). The islands of the Caribbean with a few exceptions are free of poisonous snakes. Only Martinique and Santa Lucia have poisonous snakes. Europe has comparatively few species of native snakes. Poisonous snakes in Europe tend to be spotty in distribution, especially towards the north. All the European poisonous snakes are medium-sized vipers of strikingly similar appearance. North Africa has few poisonous snake species found in the desert. This is probably due to the rigors of the desert climate. The majority of snakes occur around zones of irrigation or natural water supply. The poisonous snake fauna of central and southern Africa is a large and diverse one. Other than sea snakes, the African poisonous snakes belong to three families, the Colubridae, the Elapidae, and the Viperidae. Africa is the only region where colubrid snakes are considered dangerously venomous. The elapids in this region can grow very large (i.e., the black mamba can attain lengths of up to 14 feet) and are some of the most dangerous snakes in existence. The vipers can also inflict dangerous bites, even though they usually do not exceed 2 feet in length. In the Near and Middle East, the vipers cause most of the snakebites. Cobras and elapids also occur in this region, but are rare or restricted in range, and inflict few bites. The number and variety of snake fauna in southeast Asia is undoubtedly the most diverse in the world. It is the only region where virtually all major groups of snakes are represented. The most important venomous snakes of the Far East are pit vipers. Cobras are important only toward the south. Most of the islands of the Pacific Ocean have no poisonous land snakes (with the exception of the Solomon and Fiji Islands),

although poisonous sea snakes can be common offshore. Australia and New Guinea have large numbers of dangerously poisonous snakes. Australia is the only continent that has more kinds of poisonous than nonpoisonous snakes. More than 60% of Australian snakes are poisonous and some are highly dangerous. Sea snakes are numerous in the Pacific and Indian Oceans and adjacent tropical and subtropical seas, but cases of serious sea snake bite are rare.

The best way to keep from being bitten by snakes is to avoid them. However, military operations may preclude total avoidance, but there are certain precautions to be taken in areas where poisonous snakes are know to inhabit.

When in snake-infested country it is important to:

a. Remember that snakes are probably more afraid of humans than humans are of snakes. Do not disturb snakes. Given the chance snakes will usually retreat to avoid an encounter. Do not step over a log is the other side is not visible. Step on it first.

b. Learn to recognize the poisonous snakes in the area of operation. Avoid killing harmless snakes.

c. Avoid walking around after dark. Many venomous snakes are nocturnal and will travel at night in search of food. Always wear boots when you walk at night.

d. Remember that snakes in general avoid direct sunlight, and that they are most active at moderate temperatures.

e. Avoid caves, open tombs, and know snake den areas. Snakes live in areas which afford protection and which may be frequented by other small animals. They may be found in considerable numbers in caves and open tombs during the winter hibernation period or during the hot summer months.

f. Remember that poisonous snakes may be found at high altitudes. Rattlesnakes are known from elevations up to 11,000 feet in the southwestern U.S. They can also climb trees and fences.

g. Walk on clear paths as much as possible. Avoid tall grass and areas of heavy underbrush or ground covering. Do not put your hands or feet in places without first looking carefully. Do not sit down without first looking around carefully.

h. Avoid swimming in waters where snakes are common. Sea snakes are not uncommon in the Indian and Pacific Oceans.

i. Avoid sleeping on the ground whenever possible. Do not put your sleeping bag near rock piles or rubbish piles or near the entrance to a cave.

j. Avoid walking close to rocky ledges. Do not turn or lift a rock or fallen tree with your hands. Move it with a stick, or with your foot if your ankle and leg are properly protected.

k. Avoid hiking/walking alone in snake-infected areas.

l. Avoid horseplay involving live or dead snakes. Snakes should not be handled carelessly. Do not handle freshly killed venomous snakes. Always carry them on a stick or in a bag if they must be returned with the patient or for identification. Teasing people with snakes may have unexpected and unfortunate results.

4. Procedures.

a. Surveillance. Snake surveillance is not commonly conducted. Occurrences of poisonous snakes are usually rare events. In fact, many species are reclusive and nocturnal and difficult to find, even if actively searching for them. However, if poisonous snakes do get inside buildings, tents, or other structures where personnel are present, they must be promptly found and eliminated.

b. Snake Guard® Snake Trap. This is a trap designed for use inside a structure where a low profile is needed. It uses specially formulated glue inside a rectangular box, which is 18 inches wide, 30 inches long and 3 inches high. The trap is of cardboard design with glue inside. This glue is applied in such a way to insure that the snakes will get caught without being able to bite or escape. The Snake Guard Trap will catch snakes up to 8 feet long without hurting them. One or two snake traps are used per room suspected of having a snake. More may be needed in large rooms. Once trapped, the snake can be destroyed or released depending upon the species and local situation. The trap design allows for snake entry from either end, after it has been placed and stabilized against a wall or other vertical surface. Place the trap along a vertical surface in areas snakes are likely to frequent, such as basements, garages, storage buildings, boathouses, stables, and other similar structures. If desired, nonpoisonous snakes can be released at a safe site. To release the snake yourself, you should first relocate the trap to a remote, shaded location away from the area of capture. After carefully opening the trap to expose the snake, pour liberal amounts of vegetable cooking oil on the snake and on the glue surrounding it. The snake will typically be able to free itself within an hour.

5. Surveillance data and action threshold.

Little if any information is available on surveillance data for poisonous snakes. No action thresholds are available. However, common sense would dictate that no poisonous snakes should be allowed to remain in areas inhabited by military personnel.

6. Preservation.

If at all possible, formalin should be used for injecting and fixing specimens. Formalin is the commercial name of a solution of formaldehyde gas (CH20) in water. It is available at drugstores and chemical supply houses in the United States at a strength of 38-40%. In Latin American countries, formalin may be purchased in many drugstores under the name "Formol" or "Formolina". Formalin must be diluted with water before it is used as a preservative. A strength of 10% formalin is best for most purposes. If the original strength is 40%, it should be mixed at a ratio of nine parts water to one part formalin. The advantages of formalin over other preservatives are: it is inexpensive, it is generally available, a small bulk of concentrated stock solution may be diluted as needed, and specimens almost never decay in it. Its principal disadvantages are: it has a very irritating odor, it is very poisonous and may cause skin irritation or rash, it has a tendency to make specimens become brittle if the solution is too strong, and tends to fade out certain colors rapidly, and it must be stored in rustproof containers.

Full strength alcohol (95% ethanol) can also be used for injection and fixing. For storage of reptiles it should be used in the proportion of 3 parts 95% alcohol to 1 part water. Specimens that have been fixed in alcohol should be carefully watched for signs of rotting. Alcoholic beverages, shaving lotions and Bay Rum contain ethyl alcohol. They should be used only in an emergency and without dilution. Liquor that is 100 proof is only 50% ethyl alcohol.

If specimens are to be made permanently immune to decomposition, it is necessary that liquid preservative be introduced into the body cavity, limbs and tail within as short a time as possible after the animals have been killed. This may be accomplished either by injection (with a hypodermic syringe) or by making deep cuts with a sharp scalpel, razor blade or scissors. The most satisfactory way is by injection. A 10-20 cc. syringe with a needle lock and several needles (gauges 18 to 26) will serve to inject most specimens.

Make a series of injections an inch or two apart through the belly into the body cavity. Begin just behind the head and continue the injections to the anus. If a syringe is not available, a series of slits must be made in the belly. For most snakes the slits should be about an inch apart and an inch long; smaller slits closer together for very small snakes. A series of slits must be made in the under side of the tail and one hemipenis everted in males. Very large snakes

may be skinned out, leaving the head and tail attached. To skin a snake make a single, long cut in the belly, just to one side of the midline, beginning about an inch behind the head and continuing to about an inch in front of the anus. Do not cut through the anal plate. Work the skin loose from the body, but do not attempt to remove the skin from the head or tail. Sever the body an inch behind the head and an inch in front of the anus, and discard the carcass. Put a strip of cloth on the inner side of the skin and roll it up, beginning at the head. Tie the roll with a piece of string and put it directly into preservative.

Small snakes may be coiled flat in the tray if the coil does not exceed three and one half inches in its outside diameter. The head should be inside. Larger snakes should be coiled in a jar and covered with preservative. If the snake has been injected it may be coiled with the belly down, tail at the bottom and head on top. If slits are used, it should be coiled with the belly up, head on the bottom and tail on top. Tall, narrow bottles should be avoided; quart and pint sizes are best. Snakes too large to coil in a gallon jar should be skinned.

After the specimens have been injected or slit, tagged, and fixed, they should be put directly into preservative. If they are to be transferred later to plastic bags for temporary storage or to be shipped they should first be allowed to remain completely immersed in preservative for at least 48 hours if formalin is used, or a week if alcohol is used. The longer they are allowed to stay in preservative, the better. They should be loose and completely covered with plenty of liquid. If space is no problem, preserved specimens are best kept in glass containers. Bail-top jars with a glass top and rubber gasket are best. Fruit jars with a metal screw top lid may be used but should be carefully watched for rust and evaporation. Glass jars with polyethylene lids and liners are more commonly used in collections, since the lids form a tight seal and are easier to obtain than the traditional bail-top jars. Metal containers should be used only for temporary storage unless coated on the inside with paraffin, "Bakvar", or some other rustproof material.

If the snake has been stored in plastic bags, simply fill up the container in which the bag is stored with wads of cloth or cotton so the bag will not knock about in transit, fasten the lid down tightly, and put the container in a wooden or heavy cardboard box for shipment. If plastic bags are not available, wrap the specimen loosely in cheesecloth and pack it carefully in a water-tight metal or plastic (polyethylene) container. If the bundle does not fill up the container, fill it up with wads of cloth or cotton. Never use paper, leaves or wood chips. Pour in enough preservative to soak the cloth. No free liquid is necessary if the specimens is well preserved.

CHAPTER 23: Snails (Schistosomiasis)

1. Military Importance.

Schistosomiasis (also known as snail fever) is a trematode parasitic disease that affects more than 200 million people in 77 countries. The public health importance of schistosomiasis is often underrated for two reasons. First, like all helminthic infections, the distribution of worms in a community is extensive but uneven. Second, severe disease usually follows after many years of asymptomatic or mildly symptomatic infection. Schistosomiasis is not militarily relevant in the sense that it is a serious "war stopper" for short contingencies. However, infections can go unnoticed until serious health implications have occurred. Most often the mild erythema at the point of entry goes unnoticed. In addition, the acute serum sickness-like disease (Katayama fever) that may occur 4-7 weeks after initial infection is rarely seen. Severe complications of this disease (cardiopulmonary, hepato-splenic, renal, central nervous system) may manifest long after the military member has redeployed to home station.

2. Background.

Humans are the definitive host for the following worms of the class Trematoda: *Schistosoma japonicum, S. mansoni, S. haematobium, S. mekongi,* and *S. malayi*. Infection takes place when cercariae, shed into fresh water by snail intermediate hosts, penetrate the skin of an individual exposed to this water. After multi-organ migration, adult worms reside in intestinal and bladder venules. Human disease is primarily associated with the host's granulomatous response to eggs retained in the tissues.

The snail is the only intermediate host of the schistosome. The snail intermediate hosts of *S. mansoni* and *S. haematobium* belong to the same family, Planorbidae, class Gastropoda. These freshwater snails have a characteristic pink or red color due to the presence of hemoglobin in their blood. The subfamily Bulininae is the major intermediate hosts for *S. haematobium* and *S. intercalatum* and is distinguishable by their ovate shells. The genus Biomphalaria serves as the intermediate host for S. mansoni and is characterized by it disk- or lens-shaped shells. *Oncomelania hupensis* is the snail intermediate host for *S. japonicum*. These snails have 6-8 left-to-right spirals and their length is about that or a rice grain. The shape is that of a small screw; hence the name "screw snail". These snails are amphibious rather than purely aquatic and tend to be confined to stable marshy habitats with constant high humidity. Snail activity is the highest in March, April, and May and again in September and October. Very little activity occurs from December to February.

3. Geographic distribution and habitat.

An estimated 500-700 million people in 77 countries are believed exposed to infection and about 200 million are believed to be infected. The geographic distribution of schistosomiasis is confined to an area between 36^0 North and 34^0 South latitude, where freshwater temperatures average 25^0 to 30^0 C. In general, this includes (1) *S. japonicum*, which is transmitted only in China, Indonesia, and the Philippines by the snail *Oncomelania hupensis*; (2) *S. mansoni*, which is transmitted by member of the *Biomphalaria* snail genus in 53 countries form the Arabian peninsula, much of Africa, Brazil, Suriname, Venezuela, and some Caribbean Islands; and (3) *S. haematobium*, which is transmitted by members of the *Bulinus africanus* snail group in 52 countries in sub-Saharan Africa, by tetraploid members of the *B. tropicus/tuncatus* complex in the Mediterranean region and Southwest Asia, by members of the *B. forskalii* snail group in Arabia and Mauritius, and by all three snail groups in West Africa.

The natural pattern of snail distribution is in the plains of water network areas, mainly on the longitudinal or transverse crossroads of rivers, irrigation ditches, and electric irrigation canals, and the lakes, pools, low swamps, and banks that are connected with them. Sometimes, because humans in their daily work carry them, snail-infested points may occur that are not connected with rivers or ditches. River snails are mainly distributed on the two banks 1 meter above or below water level. They are rarely found below that level. The snails are most densely populated nearer to the water level. The general rule is the less the slope of the bank, the wider the distribution of snails. Snails in irrigation ditches and canals are mostly distributed in a line on the water level. Some snails also exist in the bed of the ditches and canals where they are easily found when the water level is low. Snails in rice fields are mostly limited to 1-2 meters near the irrigation gutters. The distribution of snails in lakes and pools is the same as in the rivers and streams they connect to. Generally, no snails live in stagnant pools.

4. Procedures.

Spring and autumn are the best seasons for snail surveillance. Rivers are the main breeding places of snails in water network areas. Irrigation canals and rice fields are also important sources of snail infestations. Concentrate your efforts to the water line when surveying rivers and ditches. When examining rice fields, investigate the inlet and outlet ditches first, then investigate the edges of the fields and finally the center of the fields.

a. River bank estimation method. The estimation method is used to calculate snail-infested areas on rivers, ditches and pools. One meter of bank length is considered at 1 m^2. Snail distributions of less than 10 per m^2 are considered low. Snail distributions of 10-20 per m^2 are considered moderate and distributions of greater than 20 per m^2 are considered high. If snails are

found in one section of a rice field, then the whole field is considered snail-infested.

b. Equal distance basket placement method. This method is most often used to evaluate the results of snail elimination campaigns. A 1-ft^2 basket (or box) is placed at each 10 m or 20 m distance of the area to be surveyed. If snails are found in the basket, then all snails in the basket, and the number of snail-containing baskets are counted to calculate the average density of living snails. The number of snails reflects the snail density. The calculation formula is:

$$\text{Average density of living snails (no./ft}^2\text{)} = \frac{\text{Total number of snails caught}}{\text{Number of baskets investigated}}$$

$$\text{Percentage of baskets with snails} = \frac{\text{Number of baskets with snails X 100}}{\text{Number of baskets investigated}}$$

5. Surveillance data and action threshold.

All bodies of freshwater in schistosomiasis endemic areas should be suspect and assumed to have intermediate host snails in them. Troops should be warned to avoid contact with all environmental freshwater sources whenever possible. The observation of one suspect snail in a body of water should render the entire body of water as being infested.

6. Preservation.

Preserve all snails that are collected in 80% ethanol.

SELECTED REFERENCES

Armed Forces Pest Management Board (AFPMB). 2001. Technical Information Memorandum 6. Delousing Procedures for the Control of Louse-Borne Disease During Contingency Operations. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

Armed Forces Pest Management Board (AFPMB). 2000. Technical Information Memorandum 19. Catalog of DoD Pest Management Material Other Than Pesticides. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 1987. Technical Information Memorandum 23. A Concise Guide for the Detection, Prevention, and Control of Schistosomiasis in the Uniformed Services. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 2000. Technical Information Memorandum 24. Contingency Pest Management Pocket Guide. Washington, (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 1998. Technical Information Memorandum 26. Tick-Borne Diseases: Vector Surveillance and Control. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 2000. Technical Information Memorandum 27. Stored-Product Pest Monitoring Methods. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 2001. Technical Information Memorandum 29. Integrated Pest Management In and Around Buildings. Washington, (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 2001. Technical Information Memorandum 30. Filth flies: significance, surveillance and control in contingency operations. (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format)..

AFPMB. 1995. Technical Information Memorandum 34. Bee Resource Manual with emphasis on The Africanized Honey Bee (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 1998. Technical Information Memorandum 38. Protecting Meal, Ready-To-Eat Rations (MREs) and Other Subsistence During Storage. (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 1995. Technical Information Memorandum 40. Methods for Trapping and Sampling Small Mammals for Virologic Testing (=CDC Manual). Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. 1999, Technical Information Memorandum 41. Protection From Rodent-Borne Diseases with Special Emphasis on Occupational Exposure to Hantavirus. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

AFPMB. Military Pest Management Handbook. Washington, DC (current version is downloadable guidance available from the AFPMB web site at **http://www.acq.osd.mil/afpmb/** which can be printed in 8.5" x 11" format).

Bennett, G.W., J.M. Owens and R.M. Corrigan. 1997. Truman's Scientific Guide to Pest Control Operations, Fifth Edition. Advanstar Communications Project, Purdue University, Indiana, 520 pp.

Carestia, R.R. and L.B. Savage. 1967. Effectiveness of carbon dioxide as a mosquito attractant in the CDC miniature light trap. *Mosq. News*, 27: 90-92.

Centers for Diseases Control. 2000. Guidelines for surveillance, prevention, and control of West Nile Virus infection – United States, *MMWR*, 49: 25-28. **http://www.cdc.gov/epo/mmwr/preview/mmwrhtml/mm4902a1.htm**

Dirig, R. 1977. *Labeling and Storing and Insect Collection*, 4-H Members' Guide M-6-7, Cornell University, NY, 21 pp.

John W. Hock Company. Instructions for the SSAM light trap. Gainesville, Fl, 4 pp. **http://www.gatrorstore.com/jwhock/ins_2112.htm**

Lane, R.P. and R.W. Crosskey. 1993. *Medical Insects and Arachnids*. Chapman and Hall, London, 723 pp.

Mallis, A. 1997. *Handbook of Pest Control, Eighth Edition*. Mallis Handbook & Technical Training Company, 1456 pp.

Metcalf, R.L. and W.H. Luckmann. 1982. *Introduction to Insect Pest Management, Second Edition*. John Wiley & Sons, New York, 577 pp.

McNelly, J.R. 1989. The CDC trap as a special monitoring tool. Proc. 76th Annual Meeting of the New Jersey Mosquito Control Association, pp. 26-33. **http://www.rci.rutgers.edu/~insects/cdctrap.htm**

Military Standard 904A (MIL-STD-904A). 1984. *Guidelines for Detection, Evaluation, and Prevention of Pest Infestation of Subsistence*. Department of Defense, Washington, DC, 18 pp.

Moore, C.G., R.G. McLean, C.J. Mitchell, Nasci RS, Tsai TF, Calisher, CH, et al. 1993.Guidelines for arbovirus surveillance programs in the United States. Fort Collins, CO: U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention.
http://www.cdc.gov/ncidod/bubid/arbor/arboguid.htm

O,Malley, C.M. 1989. Guidelines for Larval Surveillance, Proc. 76th Annual Meeting of the New Jersey Mosquito Control Association, pp. 45-55.
http://www-rci.rutgers.edu/~insects/larvsurv/htm

Pecor, J. 1997. Laboratory and Field Protocols, Walter Reed Biosystematics Unit, Washington, DC, 11 pp. **http://wrbu.si.edu/www/labman/labman.html**

Peterson, A. 1964. *Entomological Techniques: How to Work with Insects*. Entomological Reprint Specialists, Los Angeles, CA, 435 pp.

Service, M.W. 1976. Mosquito Ecology Field Sampling Methods. Applied Science Publishers, London, 583 pp.

TB MED 561. 1992. *Occupational and Environmental Health Pest Surveillance*, HQ Department of the Army, Washington, DC.

Truman's Scientific Guide to Pest Control Operations, Fifth Edition. 1997. Bennett, G.W., J.M. Owens, and R.M. Corrigan, eds. Advanstar Communications, Inc. Cleveland, OH, 520 pp.

USAFSAM-SR-89-2. 1989. Ticks and Tick-borne Diseases Affecting Military Personnel. Jerome Goddard, Ed. Brooks Air Force Base, Texas. 140 pp.

United States Department of Agriculture (USDA). 1978. *Surveillance and Collection of Arthropods of Veterinary Importance,* USDA Agric. Handbook No. 518, Washington, DC, 125 pp.

USDA. 1986. *Insects and Mites: Techniques for Collection and Preservation,* USDA Misc. Publ. No. 1443, Washington, DC, 103 pp.

United States Department of Health, Education, and Welfare. 1977. *Handbook on the Prevention and Treatment of Schistosomiasis*, DHEW Publ. No. (NIH) 77-1290, Washington, DC, 172 pp.

Walter Reed Biosystematics Unit. 2001 and current. Current version is downloadable at URL **http://wrbu.si.edu**. This web page offers a wide variety of services for identification of any arthropod. Included are the means for submission of specimens, full text articles on identification of mosquitoes, and the full taxonomic catalog of the mosquitoes of the world.

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
MOSQUITOES – Family Culicidae **LARVAL COLLECTIONS**	Standing water, both permanent and temporary (swamps, ditches, pools, ponds, streams, flooded rice fields)	**Plastic Dipper** Culicines-use a quick intercepting move into the water. Anophelines – skim dipper along surface of water.	Identify all breeding sites within a 2-mile radius of the area to be protected. Sample a minimum of 10 (or 10-20%) representative breeding sites from at least 3 different locations (stations) identified during the initial survey number.	TB MED 272 TB MED 561 DA FORM 8010-R or DA FORM 8012-R
	Artificial containers (cans, barrels, tires, rain gutters, animal troughs, etc.) and natural containers (water-filled axils of bromeliad plants or cut sections of bamboo)	**Plastic Dipper** (Container breeding *Aedes* mosquitoes such as *Aedes aegypti* and *Aedes albopictus*)	Sample a minimum of 10 or 10-20% of breeding sites identified.	
	Tree holes	**Large-mouth pipette, turkey baster or siphon**	Sample a minimum of 10 tree holes or 10-20% of breeding sites identified.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
MOSQUITOES – Family Culicidae **ADULT COLLECTIONS**	Area(s) occupied by or in close proximity to personnel	**Light Trap** (CDC, SSAM) Limited to nocturnal adult mosquito species, that are attracted to light. May be used with CO_2 or other attractants (i.e., Octenol). Use of a black light trap may increase collection results.	Minimum of 3 light traps. Operate light traps 3-4 nights a week from dawn to dusk, depending on mosquito populations and risk of mosquito-borne diseases.	TB MED 561 DA FORM 8010-R or DA FORM 8011-R NEHC-TM 6250.98-2
	Dark, cool, humid places (caves, culverts, houses, stables, latrines, etc.)	**Natural Resting Stations** Use a mouth or mechanical aspirator or place a white sheet on the floor and collect mosquitoes knocked down with an aerosol pesticide (i.e., d-Phenothrin).	Sample a minimum of 5 stations per installation, either natural or artificial, with collections made at least 2 days per week.	
	Shaded, humid areas that are protected from the wind	**Artificial Resting Boxes (approx. 1 cu. ft.) –** Small wooden boxes to act as miniature enclosures. Use a mouth or mechanical aspirator or place a white piece of paper on the bottom of the box and collect mosquitoes knocked down with an aerosol pesticide (i.e., d-Phenothrin).	Minimum of 3 resting boxes per installation.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
	Indoors or outdoors depending on blood feeding behavior of suspected vector	**Protected Landing Rate Counts** CAUTION – This technique may increase the exposure of survey personnel to disease. Use a mouth or mechanical aspirator to collect mosquitoes as they land, but BEFORE they bite.	Minimum of 3 stations once every 1-2 weeks. Time landing counts depending on peak biting activity: dusk (1900-2100 hrs), night (2400-0330 hrs) or dawn (0400-0600 hrs)	
	Outdoor area(s) occupied by or in close proximity to personnel	**Animal Baited Traps.** Usually used for pathogen transmission studies. Not recommended in operational settings.		
MOSQUITOES – Family Culicidae **EGG COLLECTIONS** (*Aedes aegypti*, *Ae. albopictu*s and other container breeding mosquitoes)	Shaded areas protected from the sun and wind	**Ovitraps –** Place in areas in full or partial shade most of the day (near walls, fences, hedges, shrubs, junk piles, tires or sheltered areas).	Place one trap at each collection site. Use a minimum of 3 collection sites in separate areas. Inspect ovitraps at least weekly.	TB MED 561 DA FORM 8010-R
PHLEBOTOMINE SAND FLIES (Family Psychodidae)	Areas within a radius of 50-100 meters of sleeping areas	**Light Trap Collections** – hang traps at knee level. Maybe ineffective in the open desert.	Use a minimum of 3 light traps, operated 2-7 nights a week from dawn to dusk.	TB MED 82 TB MED 561 DA FORM 8020-R USDA Handbook No. 518

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
	Areas of moderate to high adult fly activity (i.e., in protected areas near suspected breeding habitats)	**Visual Landing Rate Counts.** Use a mouth or mechanical aspirator to collect flies as they land, but BEFORE they bite. CAUTION – This technique may increase the exposure of survey personnel to disease.	Use a minimum of 3 stations once every 1-2 weeks.	
	Larval breeding habitats	**Sticky (Oil) Paper Traps.** Place white paper covered with castor oil and rolled into a cylinder inside animal burrows, soil crevices, and rock piles. Oil traps are effective in open and dry areas.	Use a minimum of 10 traps in at least 3 different locations.	
	Near larval breeding habitats	**Animal Baited Traps.** Usually used for pathogen transmission studies. Not recommended in operational settings.	Minimum of 3 stations once every 1-2 weeks. Time landing counts depending on peak biting activity: dusk (1900-2100 hrs), night (2400-0330 hrs) or dawn (0400-0600 hrs)	
	Daytime resting sites both inside and outside dwellings	**Use a mouth or mechanical aspirator and flashlight to collect flies from resting sites.**	Sample a minimum of 10 sites.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
TSETSE FLIES (Family *Glossinadae*) Occur only in Equatorial Africa.	Variety of suspect habitats (long stream sides, water holes, wooded areas, thickets, and savanna areas)	**Protected Landing Rate Counts of Adults** –thickets around permanent pools of water or inside huts. Use a mouth or mechanical aspirator to collect flies as they land, but BEFORE they bite. CAUTION – This technique may increase the exposure of survey personnel to disease.	Usually in intervals from 5 minutes to 1 hour, depending upon fly population.	TB MED 276 TB MED 561 DA FORM 8020-R USDA Handbook No. 518.
		Vehicular or Foot Patrols. Human or vehicle moving slowly (<15 mph) and stopping at frequent intervals (every 200–500 meters). Use a mouth or mechanical aspirator to collect flies as they land, but BEFORE they bite.	Complete a minimum of 5 transects in at least 3 different habitats.	
		Fly-Rounds. Used to determine the focus and density of infestations. Standard paths along fixed compass bearings (i.e., north, south, east and west).	Collection spots are indicated every 100-200 meters along a path not to exceed 8 km. At each spot, the team collects all flies.	
OTHER BITING FLIES – Adult Collections. Horse Flies & Deer Flies (Family	Areas near aquatic and semi-aquatic larval breeding habitats.	**Protected Landing Rate Counts.** Use a mouth or mechanical aspirator to collect flies as they land, but BEFORE they bite.	Usually in intervals from 5 minutes to 1 hour, depending upon fly population.	TB MED 561 DA FORM 8020-R

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
Flies (Family Tabanidae), Black Flies (Family Simuliidae), Biting Midges (Family Ceratopogonidae), and Stable Flies and Horn Flies (Family Muscidae)	Areas occupied by or in close proximity to personnel	**Light Trap** (CDC, SSAM) Use only for biting midges. This method is NOT useful for black flies, muscids and tabanids.	Use a minimum of 3 light traps. Operate light traps between 2 and 7 nights a week from dawn to dusk, depending on biting midge populations and the risk of vector-borne diseases.	TB MED 561 USDA Handbook No. 518 USDA Misc. Publ. No. 1443
	Vicinity of breeding areas, in insect swarms, in vegetation, or flies attempting to blood feed	**Sweep Net –** Used for qualitative sampling.	Take a minimum of 3 sweep net samples.	
	Shaded areas near aquatic and semi-aquatic larval breeding habitats	**Malaise Trap.** Works well for collecting Tabanids. Use of CO_2 will increase trap efficiency.	Use a minimum of 3 days trapping per site selected.	
		Protected Landing Rate Counts. Use aspirator to collect flies as they land.	Usually in intervals from 5 minutes to 1 hour.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		Animal Baited Traps. Usually used for pathogen transmission studies. No recommended in operational settings.	Minimum of 3 stations once every 1-2 weeks. Time landing counts depending on peak biting activity: dusk (1900-2100 hrs), night (2400-0330 hrs) or dawn (0400-0600 hrs)	
		Sticky (Oil) Paper Traps. Place trap (white paper covered with castor oil) 12-18 inches above the ground in the immediate breeding area.	Use 3-6 traps in at least 3 different locations.	
	Over the body of water to be sampled	**Adult Emergence Traps.** May be used to sample adult insects emerging from the larval habitat.	Use a minimum of 1 trap at 3 different locations.	
OTHER BITING FLIES – Larval Collections. Horse Flies & Deer Flies (Family Tabanidae), Black Flies (Family Simuliidae), Biting Midges (Family Ceratopogonidae),	Breeding site	**Flotation with Salts.** Use concentrated solution of MgSO4 to “float” larvae to the surface of a container where they can be removed.	Use a minimum of 10 samples from 3 different locations.	USDA Handbook No. 518

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
and Stable Flies and Horn Flies (Family Muscidae)				
FILTH FLIES – Adult Collections. House flies (Family Muscidae), Eye gnats (Family Chloropidae)	Landfills, stables, kennels, refuse collection points, latrines, etc.	**Visual Counting Flies on Resting Sites.** Conduct weekly surveys throughout the fly breeding season. Use Fly Grill technique or Fly Bait technique (if indoors).	Minimum of 3 separate count periods used at 3 different locations.	AFPMB TIM No. 30 TB MED 561 DA FORM 8015-R USDA Handbook No. 518
		Light Trap (CDC, SSAM) Use with ultraviolet (UV) lamp. May be used with CO_2 or other attractants (i.e., octenol)	Minimum of 3 light traps. Operate light traps between 2 and 7 nights a week from dawn to dusk, depending on mosquito populations and risk of mosquito-borne diseases.	
		Sticky Traps. Conduct weekly surveys throughout the fly breeding season.	Exposure sticky traps to flies for a period of 24 hours. Minimum of 1 sticky trap at 3 different locations.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		Live Traps. Recommended only when live specimens are required for identification or resistance testing.	Exposure live traps to flies for a period of 24 hours. Minimum of 1 live trap at 3 different locations.	
		Sweep Netting. Useful for collecting live flies for identification, but not very quantitative.	Minimum of 10 sweep net samples from 3 different locations.	
MYIASIS-PRODUCING FLIES – Larval Collections. Bot Flies and Warble Flies (Family Oestridae), Rodent Bot Flies (Family Cuterebridae), Blow flies (Family Calliphoridae), Flesh Flies (Family Sarcophagidae)	Host animals	**Visual Examination of Host Animals.** Collect larvae directly from body openings or "express" them from dermal cysts.	Examine a minimum of 10 animals of each species from 3 different locations.	AFPMB TIM No. 30 USDA Handbook No. 518
FLEAS *Pulex irritans, Xenopsylla cheopis, Nosophyllus fasciatus* and many	Inside dwellings or work areas	**Sticky Flypaper** wrapped (sticky-side-out) around the legs of person on outside of pants. Method is used indoors.	Walk around room for 1 minute and count number of fleas on sticky paper.	USDA Handbook No. 518

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
fasciatus and many others		**White Cloth on Floor.** Collect fleas as they jump or crawl on cloth.	Count number of fleas on cloth every minute for at least 10 intervals.	
	Host animal burrows and habitats	**Soapy water.** Place dead rodents in soapy water solution. Fleas can be collected and strained from soapy solution.	At least 10 rodents from 3 different locations.	
		Swabbing device. Use a flexible stick with flannel cloth attached to end to "swab" all sides of animal burrows.	At least 10 rodent burrows from 3 different locations.	The Biology of Disease Vectors
	Infested homes and living quarters	**Sifting and Flotation of Dust and Debris from Infested Homes.**	Minimum of 10 samples from 3 different locations.	
	Outdoor or indoor environments	**Visual counting of fleas.** Count the number of fleas landing or crawling in 1 minute on the lower parts of the legs of the observer. Caution: the trouser legs should be tucked into the socks to prevent bites.	Make a minimum of three 1-minute flea counts at a minimum of 3 different locations.	South Central Asia DVEP
SUCKING LICE (Order Anoplura)	Host specific	**Visual Inspection of Host.** Depends on meticulous examination of the hair, clothes of people, or wool of the host animals. Grossly infested animals may have large areas	Minimum of 10 host animals from 3 different locations. Critically examine a representative sample of the herd.	USDA Handbook No. 518

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		of skin that become raw, bruised, and denuded of hair as a result of constant rubbing.		
BED BUGS (Family Cimicidae)	Inside human dwellings in cracks and crevices	**Observation** of fecal spotting on walls, headboard of bed, and furniture. Pick from crevices in walls or other hiding places.	Sample a minimum of 10 dwellings by visually inspecting with a flashlight. Spend at least 0.5 person-hours per dwelling for each survey.	
	Inside human dwellings	**"Sweet Sickly" Odor.**	Sample a minimum of 10 dwellings	
ASSASSIN BUGS, KISSING BUGS (Reduviidae)	Resting habitats in and directly adjacent to human dwellings	**Direct Observation of Domestic and Peridomestic Habitats.** Collect from domestic (wall crevices, thatch roofs, bed frames, mattresses, posters) and peridomestic (piles of tiles, firewood, inside chicken-houses, near cattle) habitats.	Sample a minimum of 10 dwellings by visually inspecting with a flashlight. Spend at least 0.5 person-hours per compound for each survey.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		Pyrethroid (Flushing) Sprays. Excitorepellent flushes bugs from hiding places. Collect from domestic (wall crevices, thatch roofs, bed frames, mattresses, posters) and peridomestic (piles of tiles, firewood, inside chicken-houses, near cattle) habitats.	Sample a minimum of 10 dwellings by visually inspecting with a flashlight. Spend at least 0.5 person-hours per compound for each survey.	
COCKROACHES Suborder: Blattaria	**INSIDE HUMAN DWELLINGS IN CRACKS AND CREVICES**	**Monitoring Traps.** Sticky and jar traps.	Place traps in or near likely harborages for a minimum of 1 night. Minimum of 10-15 sticky traps or 6 jar traps at different locations for a dining facility kitchen.	TB MED 561
		Visual Surveillance. Day time surveillance with normal flashlights or night time surveys using red lens flashlights.	Inspect all likely harborage areas in a facility.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		Pyrethroid (Flushing) Sprays. Excitorepellent flushes cockroaches from hiding places.	Spray in key harborage areas in a facility for a survey. Spray all likely harborage areas if flushing as part of a control program.	
		Vacuuming can be used to survey and control cockroaches. This method is very efficient and effective. CAUTION: vacuum must be fitted with a HEPA filter.	Sample at least 3 different areas within each room to be surveyed.	
STORED-PRODUCT PESTS	Inside or outside of warehouses & subsistence storage areas	**Pheromone/Food Attractant Trap (PFAT).** Pinpoints the location of an infestation.	Trap Density **Indian Meal Moth** - 1 trap per 25,000 cu. ft.. **Beetles** - arrange traps in grid pattern at 25-50 ft. intervals. Check traps weekly. Do not place traps within 30 ft. of exterior doors.	AFPMB TIM No. 27
		Rodent glue boards and/or cockroach traps.	Need higher density of placement than PFAT.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		Light Traps. Place 6 ft. above the floor. Will not collect saw-toothed grain beetle or other "non-flying stored-product insects.	Use at least 3 light traps per warehouse or 1 light trap in smaller storage areas.	
HARD TICKS (Family Ixodidae)	Tick habitat – Select several different edge habitats (interface areas between grassy and wooded areas) in areas of high mammal activity	**Tick Drags/Tick Flags** Use white, soft cloth. Do NOT perform in rain or on wet vegetation such as after rainfall or in the early morning.	Use 100 meter transects, checking for ticks every 10 paces. Minimum of three transects at 3 different habitats.	AFPMB TIM No. 26 DA FORM 8016-R USAFSAM-SR-89-2
		Tick Walks Wear white, 100% cotton clothing or coveralls, OR surgical stocking net pulled over the legs of the BDU. Do NOT perform in rain or on wet vegetation such as after rainfall or in the early morning.	Use 100 meter transects, checking for ticks every 10 paces. Minimum of three transects at 3 different habitats.	AFPMB TIM No. 26 DA FORM 8018-R
		CO_2 (Dry Ice) Traps Can yield the most ticks per man-hour expended. Use ¼ - 1 LB block of CO_2 per trap.	Minimum of 3 traps used over 24 hour period at 3 different locations.	AFPMB TIM No. 26 DA FORM 8017-R USAFSAM-SR-89-2
		Host Trapping and Examination May provide most accurate assessment of a local tick population when appropriate hosts are sampled. See RODENT section.		AFPMB TIM No. 26 DA FORM 8019-R

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
	Inside animal dens and caves. This method will only work for species that live in burrows and caves (e.g., *Ornithodoros parkeri*)	**Burrow Swabbing.** Insert cotton flannel cloth wrapped around a stick into animal den. Retrieve cloth and inspect for ticks.	Minimum of 10 dens at a variety of different locations.	
SOFT TICKS (Family Argasidae)	Nesting sites of hosts or hiding places	**Hand Picking in Tick's Natural Habitat.** Crudest method of survey.	Minimum of 10 sites at 3 different locations.	USDA Handbook No. 518
	Inside animal dens and caves	**Burrow Swabbing.** Insert cotton flannel cloth wrapped around a stick into animal den. Retrieve cloth and inspect for ticks.	Minimum of 10 dens at a variety of different locations.	The Biology of Disease Vectors
PARASITIC MITES (*Psoroptes, Sarcoptes, Chorioptes, Otodectes, Notoedres, Knemidocoptes*) and	Lesions of hosts.	**Skin Scrapings. Skin scrapings.** Scrape suspect areas and preserve or mount on microscopic slide.	Take are least one skin scrapping from host. However, 2 skin scrapping samples from different areas is recommended.	USDA Handbook No. 518
	Visible on host animal. Bedding, nests, and associated debris should also be examined.	**Aspirator or Camel-Hair Brush.**	Minimum of 10 sites at 3 different locations.	
CHIGGER MITES (*Leptotrombidium*)	Chigger habitat	**12-inch Black Squares.** Place 12-inch square black paper	Minimum of 10 squares in at least	South Central Asia DVEP

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
		or plastic on the ground in suspected chigger habitat for 1-5 minutes.	three different sites.	
Astigmatid Mites (dermatitis and allergic conditions)	Dust indoors from carpets, couches, drapes, and padded furniture	**Vacuum sampling of dust.**	Density of mites is expressed as mites per gram of dust examined.	The Biology of Disease Vectors
VENOMOUS ARTHROPODS - Spiders, Bees, Wasps, Fire Ants, Centipedes, Millipedes, and Scorpions	Typical habitats and nests.	**Observation of typical habitats**	Observe a minimum of 10 habitats within the suspected area.	TB MED 561 DA FORM 8020-R
		Pitfall or Dish Traps. May be baited with various substances depending on intended species.	Minimum of 3 days trapping at 3 different locations. Inspect traps daily.	USDA Misc. Publ. No. 1443
		Sugar Bait – Works well for bees and wasps.	Minimum of 3 days trapping at 3 different locations. Inspect traps daily.	USDA Misc. Publ. No. 1443
RODENTS AND OTHER MAMMALS	Inside and around outside of buildings	**Commensal rodent visual survey.** Look for the following signs: Sounds, droppings, urine, rub marks, runs and trails, tracks, odor, gnawing, burrows, nests, and food caches.	Survey a wide variety of sites (at least 10) in and around any buildings suspected of harboring rodents. This survey should be conducted carefully, using a flashlight.	TB MED 561

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
	Near brush piles, fallen logs, burrows, abandoned debris and other areas that provide shelter	**Live Traps** – Best method if collecting rodent blood samples and ectoparasites. Set traps out before dark. **Snap traps** – Not useful for collecting rodent blood samples or collecting ectoparasites. Set traps out before dark and collect at dawn.	Minimum of 50 traps spread over 3 different locations. Place traps in lines of 10-20 traps at approximately 5-meter intervals.	AFPMB TIM No. 40 DA FORM 8019-R
BIRDS AND BATS	Inside (attics) and outside abandoned structures and living quarters	**Direct Observation.**	Survey a wide variety of locations in and around the suspected structure.	
POISONOUS SNAKES	Inside and outside work and living areas	**Direct Observation**	Usually active surveillance for snakes is not conducted; often troops will inform surveillance personnel that a snake has been seen in the area.	
	Inside buildings where snakes are a problem or a potential danger to personnel	**Snake Guard® Snake Trap**	One or two snake traps are used per room suspected of having a snake. More may be needed in large rooms.	

APPENDIX 1. QUICK GUIDE TO VECTOR AND PEST SAMPLING				
PEST SAMPLED	**PHYSICAL LOCATION(S)**	**TYPE OF SAMPLING**	**SAMPLE SIZE**	**SAMPLING DOCUMENT (REFERENCE)**
FRESHWATER SNAILS	Plains of freshwater network areas (rivers, irrigations ditches, rice fields, lakes, pools, low swamps)	**River bank estimation method.** Count number of live snails per 1 meter of bank length.	Make a minimum of 3 counts per body of water within one meter of the water line.	
		Equal distance basket placement method. Use a 1-ft^2 basket or box every 10-20 meters distance for the area to be surveyed.	Make a minimum of 3 counts per body of water within one meter of the water line.	

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
MOSQUITOES (Family Culicidae)	**Malaria**	*Plasmodium falciparum* *Plasmodium vivax* *Plasmodium ovale* *Plasmodium malariae*	*Anopheles* spp.	Worldwide; 64^0 North to 32^0 South latitude in tropical, sub-tropical, and temperate areas.
	Filariasis	*Wuchereria bancrofti*	*Culex pipiens pallens* *Culex quinquefasciatus* *Anopheles gambiae* *Anopheles funestus* *Anopheles farauti* *Anopheles punctulatus*	Africa, Asia, Central America, South America, Pacific Islands
		Brugia malayi	*Mansonia uniformis* *Mansonia annulifera* *Anopheles sinensis* *Anopheles campestris* *Aedes togoi*	Asia, India, Sri Lanka
		Brugia timori	*Anopheles barbirostris*	Isolated focus in Indonesia
	Dengue and Dengue hemorrhagic fever	Family Flaviviridae, *Flavivirus*, types 1 to 4	*Aedes aegypti* *Aedes albopictus* *Aedes polynesiensis*	Old and New World Tropics, South Pacific

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Yellow fever	Family Flaviviridae, *Flavivirus*	Urban *Aedes aegypti* Sylvatic *Aedes simpsoni* *Aedes africanus* *Aedes furcifer* *Aedes luteocephalus* *Haemogogus* spp. *Sabethes* spp.	South America and tropical Africa
	West Nile fever (WN)	Family Flaviviridae, *Flavivirus*	*Culex quinquefasciatus* *Culex pipiens* *Culex tritaeniorhynchus* *other Culex* spp. *Aedes albopictus* Ticks	Africa, Europe, eastern USA, India, Russia, Pakistan Russia
	Japanese encephalitis (JE)	Family Flaviviridae, *Flavivirus*	*Culex vishnui* *Culex tritaeniorhynchus* *Culex gelidus*	Southeast Asia, China, Japan, South and North Korea, India
	Murray Valley encephalitis (MVE)	Family Flaviviridae, *Flavivirus*	*Culex annulirostris* *Aedes tremulus*	Australia
	St. Louis encephalitis (SLE)	Family Flaviviridae, *Flavivirus*	*Culex pipiens* *Culex nigripalpis* *Culex tarsalis*	North and South America from southern Canada to Argentina
	Rocio fever	Family Flaviviridae, *Flavivirus*	*Aedes* spp. *Psorophora ferox*	Brazil
	Ross River fever	Family Togoviridae, *Alphavirus*	*Aedes* spp. *Culex* spp. *Anopheles* spp. *Mansonia* spp.	Australia and South Pacific islands

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Eastern equine encephalomyelitis (EEE)	Family Togoviridae, *Alphavirus*	*Culiseta melanura* *Culex spp.* *Ochlerotatus spp.*	North America, South and Central America
	Western equine encephalomyelitis (WEE)	Family Togoviridae, *Alphavirus*	*Culex tarsalis* *Ochlerotatus melanimon* *Ochlerotatus dorsalis* *Culex* spp.	Western North America
	Venezuelan equine encephalitis (VEE)	Family Togaviridae, *Alphavirus*	*Psorophora* spp. *Aedes* spp. *Culex* spp.	North America, South and Central America
	Sindbis fever	Family Togaviridae, *Alphavirus*	*Culex univittatus* *Culex neavei* other *Culex* spp.	Europe, Africa, Asia and Australia
	O'Nyong-nyong Fever	Family Togaviridae, *Alphavirus*	*Anopheles gambiae* *Anopheles funestus*	Tropical Africa
	Chikungunya fever	Family Togaviridae, *Alphavirus*	*Aedes aegypti* *Aedes albopictus* *Aedes vittatus* *Mansonia* spp.	Africa and Asia
	Barmah Forest virus	Family Togaviridae, *Alphavirus*	*Culex annulirostris* *Aedes* spp. *Coquillettidia* spp.	Australia
	Semliki Forest virus	Family Togaviridae, *Alphavirus*	*Aedes* spp. *Anopheles funestus*	Sub-Saharan Africa
	California encephalitis	Family Bunyaviridae, *Bunyavirus*	*Ochlerotatus melanimon* *Ochlerotatus dorsalis*	California, USA
	Bunyamwera virus	Family Bunyaviridae, *Bunyavirus*	*Aedes* spp. *Mansonia* spp. *Culex* spp.	East, West and Central Africa
	Cache Valley virus	Family Bunyaviridae, *Bunyavirus*	*Aedes* spp. *Psorophora* spp. *Anopheles* spp.	North America

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Jamestown Canyon virus	Family Bunyaviridae, *Bunyavirus*	*Aedes* spp. *Culiseta inornata*	North America
	La Crosse encephalitis virus	Family Bunyaviridae, *Bunyavirus*	*Ochlerotatus triseriatus* *Ochlerotatus hendersoni*	North America
	Snowshoe hare virus	Family Bunyaviridae, *Bunyavirus*	*Aedes* spp.	North America
	Tahyna virus	Family Bunyaviridae, *Bunyavirus*	*Aedes vexans* *Aedes caspius* *Aedes cinereus* Culex spp.	Central Europe and Asia
	Trivittatus virus	Family Bunyaviridae, *Bunyavirus*	*Ochlerotatus trivittatus* *Ochlerotatus triseriatus*	USA
	Rift Valley Fever	Family Bunyaviridae, *Phlebovirus*	*Anopheles* spp. *Culex pipiens* *Aedes* spp. Simuliidae (black flies) *Culicoides* (biting midges)	Africa
	Bovine ephemeral fever	Family Reoviridae, *Ephemerovirus*	*Culex spp.* *Aedes spp.* *Culicoides* (biting midges)	Africa, Middle East, Asia, and Australia
SAND FLIES Family Psychodidae	Leishmaniasis	*Leishmania* spp.	Old World *Phlebotomus* spp. New World Lutzomyia spp.	Old World and New World tropics
	Sand Fly Fever	Family Bunyaviridae, *Phlebovirus*	*Phlebotomus papatas* *Phlebotomus pernicious* *Lutzomyia* spp.	Worldwide; common in Europe, Africa, central Asia and the New World tropics

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Bartonellosis	*Bartonella bacilliformis*	*Lutzomyia verrucarum*	South America only in western Andes mountains
BITING MIDGES Family Ceratopogonidae	Mansonellosis (filariae)	*Mansonella perstans* *Mansonella streptocerca* *Manonella ozzardi*	*Culicoides* spp.	Western and central Africa and the American tropics
	Nairobi sheep disease	Family Bunyaviridae, *Nairovirus*	*Culicoides tororensis*	East Africa and Somalia
	Oropouche virus	Family Bunyaviridae, *Phlebovirus*	*Culicoides paranensis*	Amazon basin of South America
	Rift Valley fever	Family Bunyaviridae, *Phlebovirus*	*Culicoides* spp.?	Africa
BLACK FLIES Family Simuliidae	Onchocerciasis	*Onchocerca volvulus*	Tropical Africa *Simulium damnosum* *Simulium naevi* *Simulium yahense* *Simulium sirbanum* *Simulium dieguerense* Central & South America *Simulium ochraceum* *Simulium metallicum*	Tropical central and west Africa, Yemen Mexico, Guatemala, and northern South America
	Mansonellosis (filariae)	*Mansonella ozzardi*	*Simulium rugglesi* *Simulium anatinum*	Western and central Africa and the American tropics

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
TSETSE FLIES Family Glossinidae Genus Glossina	African Trypanosomiasis "African Sleeping Sickness"	*Trypanosoma brucei gambisiense* *Trypanosoma brucei rhodesiense*	West Africa *Glossina palpalis* *Glossina tachinoides* *Glossina fuscipes* *Glossina pallicera* *Glossina caliginea* East Africa *Glossina morsitans* *Glossina swynnertoni* *Glossina longipalpis* *Glossina pallidipes* *Glossina austeni*	Sub-Saharan Africa and Zanzibar
DEER FLIES Family Tabanidae	Loiasis	*Loa loa*	*Chrysops silacea* *Chrysops dimidiata* *Chrysops distinctipennis*	Tropical west and central Africa
	Anthrax	*Bacillus anthracis*	*Chrysops* spp.	Worldwide
	Tularemia	*Francisella tularensis*	*Chrysops discalis*	Worldwide
EYE GNATS Family Chloropidae	Pinkeye (Conjunctivitis)	*Streptococcus pyogenes*	*Hippelates* spp. *Siphunculina* spp.	Worldwide
	Yaws	*Treponema pallidum*	*Hippelates* spp. *Siphunculina spp.*	West Africa, Central America, India, Southeast Asia and South Pacific islands
MYIASIS-PRODUCING FLIES Family Calliphoridae Family Sarcophagidae	Human Bot fly, torsalo	Myiasis (obligate)	*Dermatobia hominis*	Mexico, Central and South America
	New World primary screwworm	Myiasis (obligate)	*Cochliomyia hominivorax*	North America, Central and South America
	New World secondary screwworm	Myiasis (facultative)	*Cochliomyia macellaria*	North America, Central and South America

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Old World primary screwworm	Myiasis (obligate)	*Chrysomya bezziana*	Africa, India, and Pacific and Indian Islands
	Old World secondary screwworm	Myiasis (facultative)	*Chrysomya rufifacies*	Europe, Asia, and Australia
	Screwworm	Myiasis (obligate)	*Wohlfahrtia magnifica* *Wohlfahrtia vigil*	Palearctic Region Nearctic Region
	Screwworm	Myiasis (obligate)	*Cordylobia anthopophaga*	North and Central Africa
	Screwworm	Myiasis (obligate)	*Cordylobia rodhaini*	North and Central Africa
	Congo floor maggot	Sucks blood	*Auchmeromyia senegalensis*	North and Central Africa
KISSING BUGS Family Reduviidae	American Trypanosomiasis, Chagas Disease	*Trypanosoma cruzi*	*Rhodnius prolixus* *Triatoma brasiliensis* *Triatoma dimidiata* *Triatoma infestans* *Panstrongylus megistus*	Central and South America
BED BUGS Family Cimicidae	No known pathogens are transmitted	Parasitism	*Cimex lectularius* *Cimex hemipterus*	Worldwide
SUCKING LICE Family Pediculidae	Louse-borne (epidemic) typhus	*Rickettsia prowazekii*	*Pediculus humanus humanus*	Africa, the Americas, and Asia
	Trench fever	*Bartonella quintana*	*Pediculus humanus humanus*	Possibly worldwide, but rare
	Epidemic (Louse-Borne) Relapsing Fever	*Borrelia recurrentis*	*Pediculus humanus humanus*	Worldwide
	Head lice	Parasitism	*Pediculus humanus capitis*	Worldwide

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Body lice	Parasitism	*Pediculus humanus humanus*	Worldwide
FLEAS Family Pediculidae	Flea-borne (murine or endemic) typhus	*Rickettsia typhi* (= *Rickettsia mooseri*)	*Xenopsylla cheopis* *Ctenocephalides felis* *Leptopsylla segnis* *Polyplax spinulosa*	Tropical, subtropical, warm temperate regions
	Plague	*Yersinia pestis*	*Xenopsylla cheopis* *Xenopsylla brasiliensis*	Worldwide
	Tungiasis, chigger, chigoe	Parasitism	*Tunga penetrans*	Tropical and subtropical regions of the Americas, West Indies and Africa

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
COCKROACHES Family Blattidae Family Blattellidae Family Blaberidae	Viruses	*Poliomyelitis*	*Blatella germanica* *Periplaneta Americana* *Supella longipalpa*	Worldwide
	Bacterial food poisoning, gastroenteritis, wound infection, skin infection and conjunctivitis	*Alcaligenes faecalis* *Bacillus subtilis* *Bacillus cereus* *Campylobacter jejuni* *Clostridium perfringens* *Enterobacter aerogenes* *Escherichia coli* *Klebsiella pneumoniae* *Proteus marganii* *Salmonella spp.* *Serratia marcescens* *Shigella dysenteriae* *Staphylococcus aureus* *Streptococcus faecalis* *Vibrio spp.*	*Periplaneta americana* *Periplaneta australasiae* *Blatta americana* *Blatta orientalis* *Blaberus craniifer* *Blatella germanica*	
	Molds	*Aspergillus niger* *Penicillin*	*Blatta orientalis*	
	Fungal infections	*Aspergillus niger* *Aspergillus fumigatis*	*Periplaneta americana* *Blatta orientalis*	
	Protozoans	*Entamoeba histolytica*	*Periplaneta americana* *Periplaneta australasiae* *Blatta orientalis* *Blatella germanica*	
	Hookworm	*Ancylostroma duodenale*	*Periplaneta americana*	

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Roundworms	*Ascaris* spp.	*Periplaneta americana* *Blatta orientalis*	
	Pinworm	*Enterobius vermicularis*	*Blatta orientalis* *Blatella germanica*	
	Tapeworm	*Hymenolepis sp.*	*Periplaneta americana*	
	Hookworm	*Necator americanus*	*Periplaneta americana*	
	Whipworm	*Trichuris trichuria*	*Periplaneta americana* *Blatta orientalis* *Blatella germanica*	
MITES	Scrub typhus	*Orientia tsutsugamushi*	Larval mites (chiggers): *Leptotrombidium* (=*Trombicula*) spp.	Southeast Asia and western Pacific
	Rickettsialpox	*Rickettsia akari*	*Liponyssoides sanguineus*	USA, former USSR, Korea, probably other areas
	Q fever	*Coxiella burnetii*	*Trombicula* spp.?	Worldwide
	Scabies	Parasitism	*Sarcoptes scabiei*	Worldwide
	Mite dermatitis	Dermatitis	*Trombiculid mites* *Dermanyssus* spp. *Ornithonyssus* spp. *Macronyssus* spp. *Pneumonyssus* spp. *Pymotes ventricosus* *Dermatophagoides farinae* *Dermatophagoides pteronyssinus*	Worldwide

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
HARD TICKS Family Ixodidae	Lyme disease, Lyme borreliosis	*Borrelia burgdorferi* *Borrelia burgdorferi* *Borrelia garinii* *Borrelia afzeli*	North America *Ixodes scapularis* *Ixodes pacificus* Europe *Ixodes ricinus* Asia *Ixodes persulcatus*	North America Western and Central Europe Asia
	Human Granulocytic Ehrlichiosis (HGE)	*Ehrlichia* sp.	*Ixodes scapularis* *Ixodes. ricinus*	USA, Asia and Europe
	Human Monocytic Ehrlichiosis (HME)	*Ehrlichia chaffeensis*	*Amblyomma americanum*	USA
	Rocky Mountain spotted fever	*Rickettsia rickettsii*	*Dermacentor variabilis* *Dermacentor andersoni* *Amblyomma cajennense*	North, Central, South America
	North Asian (Siberian) tick-borne typhus	*Rickettsia sibrica*	*Dermacentor marginatus* *Dermacentor* spp. *Haemaphysalis* spp.	Former USSR and Mongolia
	Human Babesiosis	*Babesia microti* *Babesia major* *Babesia divergens*	*Ixodes scapularis* *Ixodes ricinus*	USA Europe
	Colorado tick fever	Family Reoviridae, *Coltivirus*	*Dermacentor andersoni*	Rocky Mountains in USA and Canada
	Crimean-Congo hemorrhagic fever (CCHF)	Family Bunyaviridae, *Nairovirus*	*Hyalomma spp.*	Asia, Europe and North Africa
	Nairobi sheep disease	Family Bunyaviridae, *Nairovirus*	*Rhipicephalus appendiculatus* *Amblyomma variegatum*	East Africa and Somalia
	Powassan fever	Family Flaviridae, *Flavivirus*	*Ixodes* spp. Dermacentor spp.	North America and Russia

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Tick-borne encephalitis (TBE) Russian spring-summer encephalitis (RSSE)	Family Flaviridae, *Flavivirus*	*Ixodes persulcatus*	Europe, Eastern Russia and Asia
	Omsk hemorrhagic fever	Family Flaviridae, *Flavivirus*	*Dermacentor reticulates* *Dermacentor marginatus* *Ixodes apromorphus*	Western Siberia in Russia
	Kyasanur Forest disease	Family Flaviridae, *Flavivirus*	*Haemaphysalis spinigera*	Southwestern India
	Louping-ill	Family Flaviridae, *Flavivirus*	*Ixodes ricinus*	Central Europe and British Islands
	Queensland tick typhus	*Rickettsia australis*	*Ixodes holocyclus*	Northeastern Australia and Tasmania
	African tick-bite fever	*Rickettsia africae*	*Amblyomma hebraeum* *Amblyomma variegatum*	Africa
	Oriental spotted fever	*Rickettsia japonica*	*Dermacentor taiwanensis* *Haemaphysalis flava*	Japan
	Mediterranean spotted fever (Boutonneuse fever)	*Rickettsia conorii*	*Rhipicephalus* sanguineus *Haemaphysalis leachi* *Hyalomma rufipes*	Europe (Mediterranean coast), eastern Russia, Africa, Middle East, India

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
	Tularemia	*Francisella tularensis*	*Dermacentor andersoni* *Dermacentor marginatus* *Dermacentor pictus* *Amblyomma* spp. *Haemaphysalis* spp. *Ixodes* spp. Rhipicephalus spp.	Worldwide
	Q fever	*Coxiella burnetii*	*Amblyomma,* *Dermacentor,* *Haemaphysalis,* *Ixodes* spp.	Worldwide
SOFT TICKS Family Argasidae	Tick-borne (endemic relapsing) fevers	*Borrelia duttoni* (Africa) *Borrelia hispanica* *Borrelia crocidurae* *Borrelia persica* *Borrelia caucasica* *Borrelia hermsi* *Borrelia turicatae* *Borrelia parkeri* *Borrelia mazzottii* *Borrelia venezuelensis*	*Ornithodorus moubata* *Ornithodorus erraticus* *Ornithodorus erraticus* *Ornithodorus tholozani* *Ornithodorus verrucosus* *Ornithodorus hermsi* *Ornithodorus turicata* *Ornithodorus parkeri* *Ornithodorus talaje* *Ornithodorus rudis*	Africa Europe, North Africa Europe, North Africa Africa, Asia Europe, Middle East North America North America North America Central/South America Central/South America
	Q fever	*Coxiella burnetii*	*Argas* spp.	Worldwide

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
RODENTS	Hantaviral diseases: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome	Family Bunyaviridae, Hantavirus	Many rodent species.	Worldwide
		Dobrava/Belgrade	*Apodemus favicollis*	Balkans
		Hantaan	*Apodemus agrarius*	Asia, Russia
		Puumala	*Clethrionomys glareolus*	Scandinavia, Europe, Russia, Balkans
		Prospect Hill	*Microtus pennsylvanicus*	North America
		Sin Nombre	*Peromyscus maniculatus*	North America
		Seoul	*Rattus norvegicus*	Worldwide
	Leptospirosis	*Leptospira interrogans*	Rats of various species	Worldwide
	Q Fever	*Coxiella burnetii*	Various species	
	Salmonellosis	*Salmonella spp.*	Various species	
	Rat bite fever	*Streptobacillus moniliformis*	Various rat species	
TREMATODES	Schistosomiasis (=Bilharziasis or snail fever)	*Schistosoma mansoni*	Freshwater snails are the intermediate host. *Biomphalaria* spp.	Africa (including Madagascar), Arabian Peninsula, Caribbean Islands, Venezuela, Surinam, French Guiana, and Brazil
		Schistosoma haematobium	*Bulinus africanus* group *Bulinus tropicus/truncates* complex *Bulinus forskalii* group	Africa (including Madagascar and Mauritius), Middle East, Portugal, India,

APPENDIX 2. QUICK GUIDE TO DISEASE PATHOGENS AND VECTORS				
VECTOR	**DISEASE**	**PATHOGEN**	**MAJOR VECTOR(S)**	**DISTRIBUTION**
			Planorbarius metidjensis	and possibly Morocco
		Schistosoma mekongi	*Ferrissia tenuis* *Tricula aperta*	Mekong River areas of Laos, Cambodia, and Thailand
		Schistosoma japonicum	*Oncomelania hupensis*	Japan, China, Taiwan, Philippines, Indonesia, and Malaya
		Schistosoma intercalatum	*Bulinus* sp.	Parts of West (Cameroon, Central African Republic, Chad, Gabon, Sao Tome and Zaire) and southern Africa
		Schistosoma mattheei	*Bulinus* sp.	Southern Africa